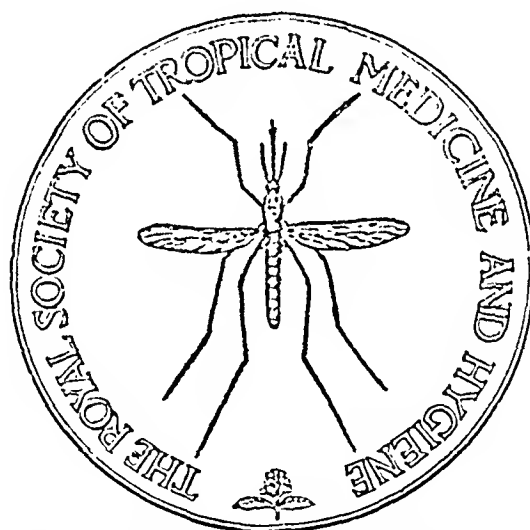


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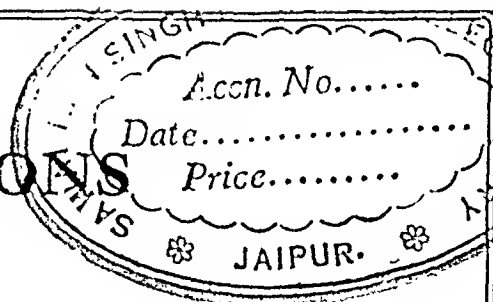


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TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

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Proceedings of a Laboratory Meeting of the Society at 8.15,
on Thursday, 21st March, 1935, at the Royal Army Medical College,
Grosvenor Road, Millbank, London, S.W.1.

Major-General Sir LEONARD ROGERS, K.C.S.I., C.I.E., F.R.S., *President*,
in the Chair.

DEMONSTRATIONS.

Dr. I. N. Asheshov.

Special culture media for the growth of bacteriophage.

Dr. G. P. Crowden.

Insulation against heat in the tropics.

The physical properties of very high reflectivity for radiant heat and very low emitting power possessed by bright metallic surfaces have been made use of for insulation against heat in tropical buildings, tentage and in sun helmets.

By means of the Moll radiation thermopile, it was demonstrated that at temperatures between 100–200° F. the heat emitted by a bright metallic surface of aluminium foil was approximately 1/10th that emitted from black, red, white or non-metallic surfaces generally. An aluminium painted surface emitted about 60 per cent. as much radiant heat as a black surface, but a layer of varnish increased its emissivity practically up to that of the black surface.

By fixing aluminium foil to reinforcing materials, such as roofing felt, canvas or thin fabrics, it has proved possible to make practical use of these properties of bright metallic surfaces. Dropped linings in tropical helmets have been shown to be more comfortable and to lower the temperature in the region of the scalp some 6° F. in the tropical sun. In tentage a second lining with aluminium foil on its outer side materially increases comfort, while in camp meat stores a loosely attached lining of the reinforced aluminium foil keeps the temperature practically at shade temperature and prevents radiation on to the foodstuffs from the non-metallic wooden roof or walls.

The division of an air space or hollow wall by a medial partition of metallic covered material increases the insulation against heat or cold to an extent equivalent to the insertion of 1 inch of cork, which has a heat insulating value equal to that of 13 inch of brick work. From this it is apparent that tropical buildings could be rendered much more habitable by the insertion of a layer of the material in the air spaces between the ceiling and roof or in hollow walls.

Dr. N. Hamilton Fairley and Colonel F. P. Mackie.

Streptothrichal ulceration of the colon with portal pyaemia.

(a) Morbid Anatomy.

1. Parts of the sigmoid and descending colon showing multiple discrete punched out ulcers in the mucosa ; from one of these a sinus passed back into a diffuse abscess in the retroperitoneal tissues which contained a thick greenish pus.

2. The liver showing extensive abscess formation in several situations. The portal system was involved (pylephlebitis) and there was also hypertrophy of the smaller bile channels.

(b) Microscopic Pathology.

Sections of the liver showing mycelial tufts of a streptothrix fungus, and also filaments of the same ramifying in the abscess wall. There was a generalised distribution of sclerotic plaques, especially around the smaller bile ducts, which did not conform to any of the common types of hepatic cirrhosis. These fibrotic areas were regarded as resulting from some chronic change of long standing and were probably independent of the more recent streptothrichal invasion. Such a condition may possibly have resulted from a past infection with *Clonorchis sinensis* or other helminthic invasion.

(c) Cultures.

Cultures, on various media, of the streptothrix derived from (1) blood during life ; (2) liver pus at autopsy.

Dr. C. A. Hoare.

A histopathological reaction of a special type on the part of the intestinal villi in ferret coccidiosis.

The ferret is known to be parasitized by two intestinal coccidia of the genus *Eimeria*, *E. furonis* and *E. ictidea* (Hoare, 1927).

While infection with the former produces no special histological changes, in the case of *E. ictidea* there is a very marked tissue reaction of an unusual type.

This parasite invades only the free end, sometimes only the summit, of the villi of the small intestine, while their basal portion remains uninfected.

When the extremity of the villus harbours the large, old forms of the coccidium (schizonts, gametocytes, oöcysts) there is evident a tissue reaction, which tends to isolate the infected portion from the non-infected one. This takes the form of an *annular constriction* which separates the two portions; it involves the epithelium and extends into the core of the villus to a depth varying with the degree of its development.

The infected portions of the villus also show the following pathological changes, which can be attributed to the pressure exerted by the constriction; dilatation of the capillaries, followed by their congestion with erythrocytes and extravasation of these elements; and, finally, focal necrosis of the infected tip of the villus, which assumes the character of a sequestrum.

A detailed, illustrated account of this process will shortly be published in the *Annals of Tropical Medicine and Parasitology*.

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Mr. B. Jobling.

An endoparasitic dipteran (*Ascodipteron* sp.) from the skin of a bat.

Three microscopical preparations, one spirit specimen and three drawings were shown illustrating the structure of this endoparasitic fly.

These flies belong to the genus *Ascodipteron*, the family Streblidae (Diptera Pupipara). Males and females of these flies newly emerged from their puparia have fully developed wings and legs. With its enormously developed proboscis the female pierces the skin of the bat which it parasitizes and drags itself into the wound, where it becomes encysted, after shedding its legs and wings. Its abdomen begins to grow round the thorax and the head, and in a short time it loses its dipterous appearance and becomes flask-shaped. In the imbedded female only the posterior end of the abdomen protrudes through the opening in the skin of the host. This parasite produces fully grown larvae which on ejection, one at a time, from the uterus drop to the ground and immediately form the puparium.

Dr. W. A. Lamborn.

The passage of leprosy bacilli through the intestine of the fly, *Musca sorbens* Wied.

The haematophagous fly *Musca sorbens* was fed on leprosy sores which contained numerous acid-fast bacilli. This fly particularly haunts man and feeds readily to repletion on sores, abrasions, or mucus from the nose or mouth. A large number of flies were fed on leprosy sores and control flies were fed on tropical ulcers which contained no leprosy bacilli.

The excreta of the flies after a feed on a leprosy sore was examined from 1 hour to 7 hours, 7 hours to 24 hours and 24 hours to 48 hours and then daily until the 7th day. Leprosy bacilli were numerous in the dejecta passed in 8 hours and they were also present in a vomit spot deposited by one of the flies 5 hours after it had fed. In excreta passed by one fly on the 7th day there were isolated acid-fast bacilli as well as acid-fast granules.

The control flies which were fed on *Ulex tropicum* on the leg of a native remained entirely negative and at no time passed acid-fast bacilli. Owing to the habits of this fly, therefore, it is obvious that it could transmit these bacilli from leprosy sores to abrasions, or to the nose or mouth of man. Slides were shown from a leprosy sore demonstrating numerous leprosy bacilli and from the dejecta of a fly 8 hours after feeding on the same sore. The dejecta showed very large numbers of leprosy bacilli.

Colonel Clayton Lane.

Distribution of periodic *Microfilaria bancrofti* in the body of a man who died at 1.15 a.m.

The microscopic sections shown were cut by Professor F. W. O'CONNOR, Columbia University, New York, from material sent him by Dr. JAMES KNOTT, St. Croix, Virgin Islands, where filariasis is periodic. The man was a cardiac case with oedema and ascites; for a week before his death he was semi-stuporous and deeply cyanotic, and for 5 weeks before that he slept fitfully for about an hour at a time. Whether periodicity of microfilariae in the blood was maintained at the time of his death is unknown.

A pre-aortic gland at diaphragm level had but little lymphoid tissue left, and had become in effect a lympho-varicocele through whose lymph vessels the products of filarial parturition could pass almost unimpeded; a similar gland a little higher up the aorta was less disorganised, and in a marginal sinus contained some microfilariae still coiled but none which had already become extended; a blood-vessel in the lung contained a mass of extended microfilariae and one which was still in the coiled state, suggesting that extended microfilariae had passed readily through the disorganised gland last noted, which however had sufficed to filter out nearly all those which were still coiled; in the kidney microfilariae lay mostly in the glomeruli, but one in an intertubular capillary was displayed for nearly its whole length; all these several embryos were sharply stained with the nuclear column well ordered.

In an adrenal gland however lay a poorly stained embryo, but the organ itself was also poorly stained; in the spleen microfilariae, which mostly lay about the capsule, showed the axial nuclear column disordered and somewhat dispersed; in the liver this disturbance and disintegration of the nuclear column was yet more marked, and here microfilariae were especially collected in areas of central necrosis where the capillaries appeared narrower than in normal parts.

Mr. H. S. Leeson and Dr. A. M. Evans.

Anopheles lesoni and forms of *A. funestus* in Southern Rhodesia.

Bred specimens of the following anophelines were shown:—*Anopheles funestus* Giles, type form (all stages), *A. funestus* var. *confusus* Evans and Leeson (all stages), *A. funestus* var. *rivulorum* Leeson (all stages except eggs), *A. lesoni* Evans (all stages), *A. longipalpis* Theobald, and captured adults of *A. funestus* var. *fuscicinctus* Leeson.

Details of distinguishing features of anatomy were illustrated by drawings; photographs of breeding places and adult haunts were also exhibited.

The uncertainty regarding the accurate identification of *A. funestus* in Southern Rhodesia led to an attempt being made to decide by a study of the early stages whether or not the various forms of *Anopheles* previously regarded as *funestus* were distinct species or at any rate varieties of that species. As a result of this work it was found that although there are certain minor variations in the adults produced, yet striking differences are associated in the external morphological features of the eggs, larvae and pupae, which are constant for each form. In the adults the males of *A. lesoni* stand alone in having three pale areas constantly present on the club of the palps, the others having only two. Other constant differences in the adults are microscopical and are found in the male terminalia and in the female pharyngeal armature. The latter character and some important differences in the egg and certain other morphological distinctions were discovered by Dr. DE MEILLON, who has done a great deal to elucidate *A. lesoni* and *A. funestus* in the Transvaal, and has kindly kept one of us informed of his findings.

With the possible exception of var. *rivulorum*, the larvae of which were found among boulders in running water, all forms appear to breed at the edges of clear, slowly moving streams shaded by vegetation; but there is still a great deal of investigation to be done both in the bionomics of these mosquitoes and in their relationship to malaria. In 1933, DE MEILLON gave evidence showing that *A. lesoni* is not a house frequenter in the Transvaal, and we understand that he is about to publish further important information regarding this species.

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Professor J. Gordon Thomson.

A microsporidian (*Octosporca*) in the intestine of *Musca sorbens* Wied.

Two species of *Octosporca* have been described, namely *Octosporca muscae-domesticae* Flu, 1911, in the epithelial cells and muscles of the midgut, hind-gut and yolk of the ovum of *Musca domestica* (Flu), *Drosophila confusa* and *D. plurilineata* (Chatton and Krempf) and *Octosporca monospora* Chatton and Krempf, 1911, in the epithelial cells of the mid-gut of *Drosophila confusa*, *D. plurilineata* and of *Homalomyia scalaris* (Brug).

In Nyasaland while carrying out feeding experiments with the haemato-phagous fly *Musca sorbens* a large percentage of certain groups of this fly had heavy infections of the gut with a microsporidian of the genus *Octosporca*. The determination of the species has not yet been completed. Flies bred in the laboratory were frequently infected. Slides were shown demonstrating the various stages of schizogony and groups of eight sporoblasts, the precursors of groups of eight spores.

Professor J. Gordon Thomson and Dr. Andrew Robertson.

Developing crescents of *Plasmodium falciparum* showing the presence of a periplastic capsule.

A series of films was shown from the peripheral blood of malignant tertian malaria cases which had occurred in Malaya, Southern India and various parts of Africa. In each instance there was a heavy infection of *P. falciparum* characterized by the presence of crescents at every stage of development. Many of these developing forms had a deeply stained band, usually pinkish or deep purple in colour, around their margins which had no relationship to the containing corpuscle and which was considered by GARNHAM (1931) to be in the nature of a capsule. It seems probable that this appearance corresponds with the formation of an ectoplasmic zone or periplast which, however, later in development, becomes less obtrusive and indeed can only be demonstrated satisfactorily in forms which have been torn during the smearing of the film. Under such circumstances the endoplasmic contents tend to pour out as a hernia-like protuberance leaving the stiffer ectoplasmic periplast or capsule more or less retaining the original spindle-shaped outline. While the presence of a capsule is readily demonstrated in ruptured crescents it is extremely difficult to detect such a structure in mature crescents. Nevertheless the characteristic spindle-shaped forms of developing female gametocytes with sharp pointed rigid extremities strongly suggest the presence of an ectoplasmic layer which enables the developing forms to retain the definite fusiform outline. So far it has not been possible to determine the presence of a periplast in developing or mature male crescents.

Proceedings of an Ordinary Meeting of the Society, held at
Manson House, 26, Portland Place, London, W.1, at
8.15 p.m., on Thursday, 16th May, 1935.

Major-General Sir LEONARD ROGERS, K.C.S.I., C.I.E., F.R.S. *President*,
in the Chair.

PAPER.

THE HUMAN ORGANISM AND HOT ENVIRONMENTS.

BY

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INTRODUCTION.

It is my purpose here to introduce a point of view. The facts with which I shall deal will be already familiar to you, but the view-point may not be quite so familiar. As you are aware, strong efforts are now being made to obtain a greater rapprochement between the more academic and the more applied aspects of medical science. From amongst the numerous problems which demand a combined attack by all medical forces, it would be difficult to select a more outstanding or more urgent example than that of the settlement of tropical lands by the Caucasian race. In this building we do reverence to the memory of the man who did so much to establish the importance of parasitism in tropical disease. Parasitology has been, and must remain the chief handmaiden of tropical medicine, but we are now in a more favourable position to consider to what extent climate *per se* influences bodily function in the tropics. I have selected the above title for this paper in order to remind you of the ways in which heat may exercise effects upon human functions, to indicate the avenues open to exploration, and to make clear the very incomplete state of our present knowledge. I hope that increasing attention will be given in the future to the physiological effects of tropical climate.

Knowledge as to the effect of heat upon the human organism may be gleaned from four sources :

1. Direct observation upon man in tropical climates.
2. Direct observation upon man under certain industrial conditions.
3. Experimental observation upon man in artificial climates.
4. Experimentation upon animals in artificial climates.

Each line of enquiry has certain advantages and disadvantages, so that a complete understanding can be obtained only by combining the evidence obtained in all four ways. At present, reliable evidence is scanty, so that only the merest of frameworks can be erected. Some of this I shall now discuss.

THE CLIMATIC ELEMENTS AND THEIR PRIMARY OPERATION.

From amongst the climatic elements which may be regarded as possessing significance for the human body, four may conveniently be considered together as the "heat" elements:

1. Radiation.—This influences the gain or loss of radiant heat by the integument.

2. Environmental Temperature.—This affects conduction of heat from, or to, the integument and respiratory tract.

3. Environmental Humidity.—This affects evaporation from the integument and respiratory tract.

4. Environmental Convection.—This modifies the loss of heat from the integument and upper respiratory tract by promoting opportunities for conduction and evaporation.

In man, the heat loss through the respiratory channels constitutes but a small fraction of the total, so that we may neglect them and focus our attention upon the integument. The "heat" elements in climate affect heat loss both directly through radiation and conduction, and indirectly through evaporation. The latter channel involves a most important process—the evaporation of water from the body surface.

When heat loss from the body by the direct route is reduced, the body temperature rises unless some compensatory mechanism can come into play to facilitate the loss and restore it to its former level. Facilitation of the direct route (radiation and conduction) can be accomplished by allowing the surface temperature to rise; facilitation of the indirect route (evaporation) can be brought about by increasing the skin surface moisture. (It should be noted that, while increased opportunities for evaporation exist in many tropical climates, these cannot be utilized unless the supply of water for evaporation is also increased; that provided by the transudation processes of "insensible perspiration" is evaporated in almost any climate.)

At first sight it may seem a relatively simple matter to enunciate how the body evokes these compensatory changes, but the further we enquire into its workings the more complex does the matter become. The structure of the body as a complex arrangement of delicate interdependent systems confers upon it two apparently paradoxical characters; it possesses a great range of adaptability to altered external circumstances, and yet adjustments in one system nearly always involve secondary changes in other systems. This is clearly seen in its adaptive reaction to heat, which may be considered in three stages:

(1) Immediate passive reactions (independent of life)—rise of skin temperature, increased evaporation of surface moisture.

(2) Primary active adaptations—cutaneous vaso-dilatation, sweating, primary water shifts.

(3) Secondary active adaptations—rise of body temperature, alterations of endocrine balance, increased pulmonary ventilation, decreased appetite.

The object of each of these reactions is the re-establishment of thermal equilibrium. Failure of the body to continue an efficient life may be due either to the impossibility of re-establishing thermal equilibrium or to adverse consequences produced incidentally in the course of reaction. I shall consider briefly the physiological mechanisms involved in each of the above reactions to heat, or secondarily to them; and then pass on to a discussion of the modes of breakdown of the equilibrium existing between the human body and its environment.

PHYSIOLOGICAL MECHANISMS INVOLVED IN ADAPTATION.

(i) *Immediate Passive Reactions.* Nothing need be said in elaboration of the obvious fact that a rise in skin temperature will facilitate heat loss by conduction and radiation and promote evaporation of any moisture present. This rise will occur passively at first as a result of decreased heat loss. An active rise may occur later.

(ii) *Primary Active Adaptations.* It appears that certain specific areas in the central nervous system are stimulated when the peripheral temperature rises. These may be given the general name of "heat regulating centres," but their anatomy and physiology is by no means as simple as the name might suggest. How a rise in skin temperature produces a stimulation of these centres is the subject of considerable controversy. Some workers maintain that the stimulation is by afferent nerves, others that a very small rise in blood temperature is sufficient for its induction. So evenly matched are the arguments advanced that one is tempted to consider that both may be true. Be that as it may, it seems quite certain that the primary adaptive reactions are brought about through the intermediation of such heat regulating centres. The chief effects produced by these centres are: (i) dilatation of the cutaneous arterioles, (ii) initiation of sweat gland activity, (iii) increase of blood volume.

(iii) *Secondary Active Adaptations.* If the above adaptations are insufficient to effect the maintenance of heat loss, the body temperature rises. This in itself is not necessarily an indication of breakdown. The body is so constituted that a moderate degree of temperature rise can be withstood without the impairment of function, and the raised temperature automatically facilitates heat loss by the various channels. We may justly regard this, therefore, as an adaptive phenomenon.

There seems to be no doubt that the excretory activity of both thyroid and suprarenal medulla is reduced in conditions of heating. Whether this depressant action is achieved through the nervous system or by a direct effect of blood temperature is not clear. By this means the metabolic activity of the whole body may actually be lowered (MARTIN, 1930) or its tendency to rise with body temperature may be compensated. The alteration of endocrine balance is probably one of the chief factors in acclimatisation.

Human pulmonary ventilation is somewhat increased as a result of heating, but this increase is of little significance in the degrees of heat naturally experienced.

Decreased appetite is a frequent accompaniment of exposure to heat. Undoubtedly this often reaches a degree which may be inimical to the organism, but a certain reduction is a truly adaptive phenomenon. The basal metabolic rate is often lower, the desire for and ability to sustain voluntary activity is diminished, and the need for metabolic response to cold is absent, so that less calorific value is required in the diet. Moreover, it must be of some advantage to the body to diminish the specific dynamic action of absorbed protein and fat. It seems to me quite wrong enthusiastically to stimulate appetite by artificial means to European consumption levels. A certain reduction is normal, further reductions are due to impaired bodily functions and the remedy is to be sought in treating the function at fault—constipation, dehydration, lack of exercise, neurasthenic disturbances, etc. Until more is known about the mechanism of appetite, it is useless to speculate upon the route by which climate affects it.

(iv) *Other Functions Secondarily Involved in the Course of Adaptation.* As I have pointed out each of the adaptations mentioned above may involve other systems and functions as a necessary or as a purely adventitious consequence. I shall mention here four of the more important :—(i) water metabolism, (ii) circulatory system, (iii) electrolyte balance, (iv) acid-base equilibrium.

The water content of the body is obviously the balance between intake and output. Under normal circumstances more is taken in than is required to meet the extra-renal losses from the body, the water-stores are full, and the excess is eliminated in the urine. When sweating occurs, this excess is drawn upon and the urine volume falls. In hot wet climates the utilisation of this normal excess may suffice if a sedentary life is pursued, but in hot dry climates, it will not suffice (the urine volume cannot fall below about 400 c.c. per 24 hours). Unless the water intake is increased, dehydration results : thirst is the normal protective device against the occurrence of dehydration. What results when dehydration progresses, we shall see later.

The circulatory system is considerably involved. The dilatation of the cutaneous vessels considerably increases the capacity of the circulation. The blood volume is increased by addition of serum from the liver and elsewhere. In this way the ratio of volume to capacity is in part prevented from falling. Should, however, dehydration or a further vascular dilatation occur, the ratio may fall and circulation be impaired. Gravity readily produces such impairment in hot conditions. Blood-pressure changes, however, are remarkably small unless failure is marked. Heart rate may be increased apart from any rise in body temperature, probably by nervous action. Once the body temperature rises, however, the rate increases very rapidly. The factors producing this are numerous. It will be readily appreciated that the cardio-vascular system is subjected to considerable demands in hot environments, from which it will be

expected that claims upon its reserves, which in normal circumstances would not be excessive, may, in these conditions, tax it beyond its resources. The possible consequences we shall consider later. Circulatory efficiency under conditions of strain, may, no doubt, be greatly enhanced by acclimatisation, as in training.

Sweat is, on the average, hypotonic, so that its loss leaves the body with an increased chloride concentration; but when water is taken to replace the sweat, the body fluids tend to develop a lowered chloride concentration. This is met by a liberation of chloride from the body stores. When these are exhausted, some extra water may be lost from the body in an endeavour to maintain the concentration. In a certain number of cases, however, continued sweating, in conjunction with copious water drinking, may lower the chloride concentration to a dangerous point. This critical event will also be considered below.

It is difficult to determine the exact state of the acid-base balance in man in such conditions as these. From the evidence available, however, it would seem that there is little essential alteration in the point of balance (although the components may be altered) unless the heating is very extreme or unless breakdown is occurring from some other cause.

THE BREAKDOWN OF EQUILIBRIUM BETWEEN BODY AND ENVIRONMENT.

As I have indicated above, external heat affects a large number of processes going on in the human body. As a result of this, the exact relationships between the body and its environment are modified, but the body can continue, with little loss of efficiency, its biological functions. In other words, the *equilibrium point* has been shifted, but there is still an equilibrium, or "steady state." As we are all aware, however, there often comes a time when something critical occurs to disturb this harmonious relationship, a cataclysm, as a result of which biological functions cannot be continued. As I pointed out early in this paper, failure to maintain this balance may be due either to the impossibility of re-establishing thermal equilibrium or to failure of some other vital function in the course of doing so. The critical events which determine these breakdowns are four: (i) hyperpyrexia, (ii) circulatory insufficiency, (iii) electrolyte imbalance, (iv) super-dehydration. Let me consider them in turn, but first let me state that I shall not attempt any distinction between purely "physiological" and so-called "pathological" events; also, that if I employ the word "physiological" in contra-distinction to "clinical," I use these words only as convenient labels for somewhat different approaches to a common problem, the one starting from the strictly normal and noting its modifications in response to heat, the other dealing with the fully developed results and endeavouring to locate the cause.

(i) *Hyperpyrexia.* This is reached when the mean temperature of the body is such that the continued life of some vital tissue is endangered thereby. In practice,

...tion, or the resistance of the body for promoting heat loss. Body temperature will rise to the critical point before thermal equilibrium between the body and its environment is re-established. Bright light shining vertically upon the head and neck may, where the mean body temperature is just sub-critical, serve by a local heating effect to raise the temperature of the central nervous system to the critical point, but apart from this local influence upon the causation of hyperpyrexia. Any factor which causes heat loss from the body (clothes, inhibition of sweating, etc.) stimulates heat production (infection, alcoholism, hyperthyroidism, drugs, severe physical work) will predispose to hyperpyrexia. In a normal resting human body in average clothing will not be sufficient to maintain a naturally occurring climate, and that one of the above factors is necessary for the production of this state. Of these, inhibition of heat loss is by far the most influential, particularly in hot dry climates; and it accounts for the frequency with which this sign is noted as hyperpyrexia.

Prognosis will predominate and affections of all levels are likely to occur. The occurrence of prodromata will depend upon the predisposing condition and the intensity of development. In general, excitatory will precede depression. Treatment needs no stressing by me. From the physical point of view, active cooling will be much more efficient than cold applications.

Insufficiency. By this I mean *a failure to maintain the blood flow at the critical temperature, without marked impairment, of the bodily functions, for a certain time.* The causation of the condition has already been discussed. When we have a circulatory system already fairly heavily taxed, and a further load is thrown upon it, either the general body or the system itself, or both are likely to suffer from circulatory insufficiency.

It is characterized by nausea, vomiting, respiratory disturbance, fatigue and dizziness, and is present in varying proportions. Heavy meals, injudicious exposure to heat, heavy exercise (especially under the stimulus of competition), dehydration and emotional disturbance are amongst the exciting causes. The exact result will depend upon the intensity of the stimulus, but the nervous system is likely always to be considerably affected. A number of sub-types are likely to be the "syncopal," the "alimentary,"

and the "cerebral." Measures for the restoration of diminished circulatory function are discussed elsewhere, but if the exciting stimulus is still present, this must be removed. In some cases may require an artificial addition to the circulating blood. For this purpose normal saline is sufficient. The addition of bicarbonate of soda is good; the addition of bicarbonate might do harm.

(iii) *Electrolyte Imbalance.* This may be defined upon empirical grounds as *the lowering of the serum chloride concentration to 100 m.equ. (mille equivalent) per litre (=365 mg. per 100 c.cm.) or less.* The correlation between such a fall of blood chloride and the onset of muscular cramp has been abundantly established, but, as far as I am aware, the mechanism of the production of the cramp has not been clearly established. I shall not, therefore, enter here into any speculations upon the subject.

The hypochloraemia is due primarily to replacement of the water lost in sweating without simultaneous replacement of the chloride. The body's defences against this occurrence are fairly readily overcome. The condition appears to be susceptible to acclimatisation. Diarrhoea and vomiting often increase the chances of a chloride deficiency.

There is often a prodromal period of one to three days with nausea, malaise, and later, tingling in the extremities. Vomiting and diarrhoea may occur. This is followed by tenderness of the extremities to movement and finally cramps. The forearm flexors and the calf muscles are most frequently affected, and the abdominal muscles are often involved. The pain of the cramp may be excruciating. Loss of weight is nearly always present. Death sometimes occurs in the untreated case, but I am not clear as to the cause of this.

The condition responds specifically to the administration of adequate amounts of chloride. This is most conveniently given as an intravenous injection of hypertonic saline. Administration can be continued orally when the more urgent symptoms subside. Injections of glucose are without effect: bicarbonate may be dangerous. Both water and salt are retained in recovery.

As a prophylactic measure, the daily excretion of sodium chloride in the urine should not be allowed to fall below 3 grammes.

(iv) *Super-Dehydration.* This may be defined as *the loss of water from the body to such an extent that continued existence is threatened, should replacement not be effected.* This will occur, of course, only when water is unobtainable. Various investigators place the critical level at different points: it probably lies at about 20 to 25 per cent. of the body weight.

I mentioned above that dehydration may be one of the factors entering into the causation of circulatory insufficiency. As dehydration progresses, symptoms of this kind become increasingly frequent, but there comes a time when much more serious results appear. The way in which super-dehydration produces its results is complex. Muscular power, circulatory efficiency, and alimentary functions, are all primarily affected. The impairment of circulation in turn produces disordered metabolism, disordered nervous function and diminished heat loss. Disordered metabolism in turn leads to an acidæmia which further affects the nervous system. This double effect upon the nervous system may be reinforced by rise of temperature. The nervous disturbance thus becomes increasingly manifest and finally passes into coma, and death. I should recommend those interested to read J. H. KING's moving description of the progress

of dehydration amongst troops lost in the waterless plains of Texas (KING, 1878).

Treatment is, of course, the restoration of body water. Vomiting and diarrhoea frequently interfere. Chloride should always be given as well. Blood transfusion may be necessary for the extreme cases.

PHYSIOLOGICAL BREAKDOWNS AND CLINICAL SYNDROMES.

After carefully considering physiological responses to heat and what I have gathered in the way of clinical information upon the syndromes which sometimes receive the general term of "heat effects," I have permitted myself five conclusions.

(1) There are four chief crises which may occur in the physiological reactions to heat :

- (a) Hyperpyrexia
- (b) Circulatory Insufficiency
- (c) Electrolyte Imbalance
- (d) Super-Dehydration.

(2) Corresponding to these four modes of breakdown there are four clinical syndromes :

- (a) Hyperpyrexia (True Heat Stroke)
- (b) Heat Exhaustion (Heat Prostration)
- (c) Heat Cramps (Miner's Cramp, Stoker's Cramp)
- (d) Dehydration.

(3) A large number of cases belong, by virtue of the great majority of symptoms, to one category, but display as well symptoms characteristic of more than one syndrome.

(4) A good deal of overlapping between syndromes in any one case is to be expected, in view of the great possible variation in the composition, intensity, and duration of the prime cause (climatic heat elements), and the wide range of reactivity possible to the exposed human organism.

(5) In the diagnosis and treatment of "heat effects," consideration should be given primarily, not to the disease category into which the case best fits, but to the causation and alleviation of individual signs and symptoms.

I shall now ask you to consider carefully and in detail Tables I, II, III and IV, each dealing with one mode of breakdown. The second column sets out the chief results to be expected from the physiological crisis named, the third column displays, for comparison, the various signs and symptoms which have been assigned to the syndrome by different authors. These are segregated into classes which bear, to my mind, different causal relationships to the essential syndrome which are indicated in the fourth column.

These tables are only intended to be illustrative and in no way pretend to be exhaustive lists of characteristics.

TABLE I.

TABLE I.

Physiological Event.	Physiological Results.	Clinical Characteristics Cited	Causal Relationship.	Clinical Syndrome.
(A) Hyperpyrexia	<p>Rise of body temperature. Nervous symptoms predominant: all levels may be involved: early excitation, late depression. Raised metabolic processes \rightarrow disordered metabolism. Thirst.</p> <p>Temperature must be reduced before any response to treatment. Prolonged hyperpyrexia may result in permanent nervous impairment. Predisposing and aggravating factors: Clothing, inhibition of sweating, infection, toxæmia, dehydration, work.</p> <p>Terminal events: rise of protein breakdown products, lactic acid, etc., in blood and urine, acidæmia.</p>	<p>Rise of body temperature. Nervous Disturbances:</p> <ol style="list-style-type: none"> 1. Irritability 2. Giddiness, Nausea, Headache 3. Convulsions 4. Stupor 5. Dyspnoea 6. Loss of reflexes. <p>Failure to respond to saline administration. Thirst.</p>	Essential	Heat Hyperpyrexia (True Heat Stroke)
		Suppressed sweating. Pre-existing toxæmia or nervous excitation. Dehydration.	Aggravating and Predisposing	
		Time for development. Inefficiency of heat-regulating mechanism.	Variable	
		Fall plasma bicarbonate		
		Rise blood lactate		
		Acidæmia	Terminal	
		Rise of non-protein N in blood		
		Alkalæmia		
		Fatigue	Accidental	
		Fall of plasma and urine Cl.	B or D	
		Cramps (as distinct from convulsions)	C	
		Diarrhoea	C	
		Fall plasma bicarbonate	C or D	Other
		Rise blood lactate	D	Coincidental
		Acidæmia	D	Heat
		Relieved by saline-bicarbonate administration	D	Effects
				D

TABLE II.

Physiological Event.	Physiological Results.	Clinical Characteristics Cited.	Causal Relationship.	Clinical Syndrome.
(B) Circulatory Insufficiency	Nervous system irritated by anoxaemia: Irritability, fatigue, faintness, etc., loss of consciousness. Muscular efficiency impaired by anoxaemia (plus commencing dehydration) Promotion of venous return by recumbency, bandaging or massage of dependent parts, or saline administration gives improvement. Cardio-vascular insufficiency. Often spontaneously reversible. Impaired alimentary functions.	Fatigue and exhaustion Headache, vertigo, etc. Sensory disturbances Fainting complex Subnormal temperature Sweating Postural effects Nausea and vomiting Responds to saline administration	Essential	Heat Exhaustion or Heat Prostration.
		Prolonged prodromata Thirst	Variable	
		Hyperpnoea Alkalaemia	Accidental	
		Pyrexia of varying degree Cramps Acidaemia	Other Coincidental Heat Effects	
		A C D		

TABLE III.

Physiological Event.	Physiological Result.	Clinical Characteristics Cited.	Causal Relationship.	Clinical Syndrome.
(C) Electrolyte Imbalance	Hypochloremia Achloruria Alkalemia Cramps and Tetany Inability to retain normal water complement	Lowered serum Cl. Responds to Cl administration Retention of Cl and H ₂ O in recovery Achloruria Loss of weight Tingling → cramps Vomiting and diarrhoea Normal temperature and pulse	Essential	Heat Cramps
		Mild pyrexia Moderate tachycardia	Accidental	
		Oliguria	D Other Coincidental Heat Effects	

TABLE IV.

Physiological Event.	Physiological Result.	Clinical Characteristics Cited.	Causal Relationship.	Clinical Syndrome.
(D) Super-dehydration	Muscular inefficiency. Reduced secretion, esp. salivary. Anorexia, dysphagia. Acidaemia Nervous system affected (i) Early (<i>Vide</i> Heat exhaustion) (ii) Later—Depression, stupor. Terminal events: Uræmia, acidaemia, rise of temperature.	Oliguria Reduced salivation Thirst Acidaemia Dyspnoea Nervous Disturbances: 1. Faintness 2. Irritability 3. Sensory Disturbance 4. Stupor Exhaustion Reduced cardiac function Anorexia, dysphagia Normal temperature Concentrated blood	Essential	Dehydration
		Uræmia Rise of temperature	Terminal	

CONCLUSION.

I have endeavoured briefly to remind you of the complexity of function in the human organism, the plurality of elements in climate affecting heat loss from the body, and the extreme interdependence of physiological systems reacting to the primary operation of these factors. That complexity of structure which makes for adaptability also imposes very definite limits upon the powers of adaptation. Failure of adaptation may be thermal, circulatory, ionic or anhydraemic. A given case may display characteristics of more than one syndrome.

I trust that these brief considerations may serve as a basis for further discussion and enquiry as to the adaptation of the body to hot environments.

I have made no attempt to give an exhaustive bibliography, but general references are given below. I must acknowledge with gratitude the interest shown, and valuable advice given me by Prof. C. LOVATT EVANS, and the stimulating enquiries of Dr. N. HAMILTON FAIRLEY. My gratitude naturally goes to the authors, too numerous to mention, from whose papers I have acquired most of the facts herein set out.

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DISCUSSION.

Professor Lovatt Evans: It is unfortunate in more ways than one that Sir CHARLES MARTIN was unable to be present this evening. First of all, because he really understands the subject and has himself done pioneer work on it, and secondly, because it was largely through Sir CHARLES MARTIN's encouragement that Dr. LEE came to England. He did this purely in order to get laboratory facilities to enable him ultimately to carry out more field work than he had hitherto been able to accomplish. We have had, I think, a remarkable paper on this complicated subject, and in a way Dr. LEE has achieved quite a feat in attempting to do something which is almost impossible, that is, to give a short account of the whole of physiology; because, if we remember, the one central point in physiological doctrine is that view, so much stressed in late years, which CLAUDE BERNARD put forward many years ago—the importance of the maintenance of the constancy of the internal environment of the body. Whenever the body is put under conditions which threaten to alter its internal environment, it responds in a great number of ways in order to oppose the tendency to change. We see very remarkable examples of this response of the body to a threatened change in its environment, in the physiological changes which take place during muscular exercise. These changes have been very thoroughly studied in several countries by a large number of workers through many years, and still there are surprising new wheels being discovered in the mechanism. It seems as though in response to muscular exercise the whole body resembles a system of cogs, and it is impossible to move any one of them without moving all the others to some extent. In like manner we see that the response to a rise of body temperature sets into operation simultaneously all kinds of reactions which tend to oppose this threatened alteration, which tends to threaten life itself ultimately. I should like to ask Dr. LEE one or two questions: one is with regard to the question of acclimatisation. Does he think, from his own actual experience in tropical climates, or from laboratory experiments which he has carried out, that acclimatisation in the physiological sense—accustoming to this surrounding heat—is really a fact? In Dr. LEE's paper there is a suggestion that one of the responses is a response of the endocrine organs—the supra-renals and thyroid—which are somewhat depressed so that basal metabolism

tends to be lowered. I think we know this even more clearly from experiments on the influence of cold surroundings, and it seems to me that more work than has been done would be quite well worth while in connection with the effect of hot climates. Evaporation of sweat from the surface of the body is extremely valuable, and we see the result in the difference of the rise of body temperature of people working in dry climates and in moist ones. Experiments in hot chambers have shown all these facts very clearly. In 1902 a case was described by Dr. ZUNTZ in a book on the physiology of marching. He described the case of a man who was frequently punished for leaving the ranks. On route marches this man used to break rank, take off his tunic and shirt, steep the shirt in water and then march on, with his tunic unbuttoned if possible. He was ultimately subjected to medical examination, and it was said that the man had no sweat glands. He replaced the evaporation of sweat by wetting his shirt, putting it on, and letting that evaporate. There is a point with regard to circulatory insufficiency, one of the fundamental points in which is apparently the fact that the circulation becomes too big for blood volume owing to loss of blood volume. There is a question of restoring this blood volume by giving injections of salt solution. I wonder if gum saline has ever been tried in place of salt solutions, where injections are required? The point in using gum saline would be that the presence of the gum would prevent the water getting out again. Dr. LEE has not really given details of the work he has done. I know he has done a very great deal, and got results which I am sure will be extremely valuable.

Sir William Willcox : First of all I would like to express my appreciation of Dr. LEE's paper. As he said, "Medicine is an applied science," becoming more and more so every day, and it is based on the pure sciences; so that if we desire the real truth about things we must go to the pure sciences—physiology, chemistry, bacteriology, physics and so on. I think this paper is admirable because Dr. LEE has approached this subject, which he admits is exceedingly difficult, from the scientific rather than the clinical side. These questions of hyperpyrexia are very important ones. A most interesting paper was read a few days ago at the Royal Society of Medicine by Dr. C. A. NEYMANN, of Chicago, on the use of artificial pyrexia in the treatment of general paralysis and various other diseases. I am interested in the "effects of heat"—I use that term in preference to heatstroke, because suddenly falling down, according to the popular idea, from the effects of heat is not often seen. I saw a large number of cases in 1917, in Mesopotamia; there were 6,242 cases of the effects of heat in the British Troops and 524 deaths. 1918 was not so hot: there were a large number in British Troops but not nearly so many: 574 cases and 31 deaths.

Dr. LEE dealt very thoroughly with the physiological causes and aspects of the effects of heat, and his remarks were very valuable. There are one or two points to which I should like to call attention. From the cases that I saw

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DISCUSSION.

Professor Lovatt Evans: It is unfortunate in more ways than one that Sir CHARLES MARTIN was unable to be present this evening. First of all, because he really understands the subject and has himself done pioneer work on it, and secondly, because it was largely through Sir CHARLES MARTIN's encouragement that Dr. LEE came to England. He did this purely in order to get laboratory facilities to enable him ultimately to carry out more field work than he had hitherto been able to accomplish. We have had, I think, a remarkable paper on this complicated subject, and in a way Dr. LEE has achieved quite a feat in attempting to do something which is almost impossible, that is, to give a short account of the whole of physiology; because, if we remember, the one central point in physiological doctrine is that view, so much stressed in late years, which CLAUDE BERNARD put forward many years ago—the importance of the maintenance of the constancy of the internal environment of the body. Whenever the body is put under conditions which threaten to alter its internal environment, it responds in a great number of ways in order to oppose the tendency to change. We see very remarkable examples of this response of the body to a threatened change in its environment, in the physiological changes which take place during muscular exercise. These changes have been very thoroughly studied in several countries by a large number of workers through many years, and still there are surprising new wheels being discovered in the mechanism. It seems as though in response to muscular exercise the whole body resembles a system of cogs, and it is impossible to move any one of them without moving all the others to some extent. In like manner we see that the response to a rise of body temperature sets into operation simultaneously all kinds of reactions which tend to oppose this threatened alteration, which tends to threaten life itself ultimately. I should like to ask Dr. LEE one or two questions: one is with regard to the question of acclimatisation. Does he think, from his own actual experience in tropical climates, or from laboratory experiments which he has carried out, that acclimatisation in the physiological sense—accustoming to this surrounding heat—is really a fact? In Dr. LEE's paper there is a suggestion that one of the responses is a response of the endocrine organs—the suprarenals and thyroid—which are somewhat depressed so that basal metabolism

tends to be lowered. I think we know this even more clearly from experiments on the influence of cold surroundings, and it seems to me that more work than has been done would be quite well worth while in connection with the effect of hot climates. Evaporation of sweat from the surface of the body is extremely valuable, and we see the result in the difference of the rise of body temperature of people working in dry climates and in moist ones. Experiments in hot chambers have shown all these facts very clearly. In 1902 a case was described by Dr. ZUNTZ in a book on the physiology of marching. He described the case of a man who was frequently punished for leaving the ranks. On route marches this man used to break rank, take off his tunic and shirt, steep the shirt in water and then march on, with his tunic unbuttoned if possible. He was ultimately subjected to medical examination, and it was said that the man had no sweat glands. He replaced the evaporation of sweat by wetting his shirt, putting it on, and letting that evaporate. There is a point with regard to circulatory insufficiency, one of the fundamental points in which is apparently the fact that the circulation becomes too big for blood volume owing to loss of blood volume. There is a question of restoring this blood volume by giving injections of salt solution. I wonder if gum saline has ever been tried in place of salt solutions, where injections are required? The point in using gum saline would be that the presence of the gum would prevent the water getting out again. Dr. LEE has not really given details of the work he has done. I know he has done a very great deal, and got results which I am sure will be extremely valuable.

Sir William Willcox: First of all I would like to express my appreciation of Dr. LEE's paper. As he said, "Medicine is an applied science," becoming more and more so every day, and it is based on the pure sciences; so that if we desire the real truth about things we must go to the pure sciences—physiology, chemistry, bacteriology, physics and so on. I think this paper is admirable because Dr. LEE has approached this subject, which he admits is exceedingly difficult, from the scientific rather than the clinical side. These questions of hyperpyrexia are very important ones. A most interesting paper was read a few days ago at the Royal Society of Medicine by Dr. C. A. NEYMANN, of Chicago, on the use of artificial pyrexia in the treatment of general paralysis and various other diseases. I am interested in the "effects of heat"—I use that term in preference to heatstroke, because suddenly falling down, according to the popular idea, from the effects of heat is not often seen. I saw a large number of cases in 1917, in Mesopotamia; there were 6,242 cases of the effects of heat in the British Troops and 524 deaths. 1918 was not so hot: there were a large number in British Troops but not nearly so many: 574 cases and 31 deaths.

Dr. LEE dealt very thoroughly with the physiological causes and aspects of the effects of heat, and his remarks were very valuable. There are one or two points to which I should like to call attention. From the cases that I saw

I came to the conclusion that autointoxication played a very great part in the symptoms in these severe cases of heat attacks. I considered the possibility that these might be due to a disturbance of the acid base equilibrium. I used to test the urine for acetone and diacetic acid, to see if there was any large excess of these bodies, but in only a small percentage of cases did this occur. I was interested when Dr. LEE said of the disturbance of the acid base equilibrium, that he did not place that high in the causation.

One other very striking point from the clinical aspect is that the effect of heat in these cases is cumulative. If a person is exposed to heat for several days he may stand it all right, and then suddenly at night an attack of hyperpyrexia occurs and death. That is what happened to Sir VICTOR HORSLEY. Though a very conscientious man, he did not obey the regulations about not going out in the sun if he had a case to see: he went whether it was the evening, morning or middle of the day, and was certainly rather indiscreet in that way. He was exposed to intense heat for several days then suddenly developed hyperpyrexia: he had three attacks, and the third proved fatal: that was an illustration of the cumulative effect of heat. We had many cases of people found dead in the morning in the hot weather where they had been exposed to heat for some time, and I used to tell the medical officers in those cases to take the rectal temperature. Sometimes in these cases of mysterious death this would be as high as 112° or 114° F. Dr. LEE has pointed out how the regulation of the body can maintain a more or less normal temperature, and how it may be taxed to the uttermost in hot weather when any intercurrent condition, such as an infection or disease, occurs to upset that capacity for maintaining an equilibrium; then you get heat hyperpyrexia. One used to see this in the hospitals. Perhaps a case of typhus, sand-fly fever or malaria, might be going on all right until suddenly hyperpyrexia occurred. I have charts of several of these cases. Of course, race plays a part. The British sent out to Mesopotamia went down with heat hyperpyrexia very easily in that hot time in 1917; but the Indians and Arabs were more adapted to it. One had to act quickly in these cases of heat hyperpyrexia. There were often no laboratory facilities and I came to the conclusion that if an Indian had heat hyperpyrexia in all probability he had some intercurrent infection, and the common one was malaria. One often did not have the opportunity of examining the blood, and it was a good rule to give an Indian case of heat hyperpyrexia an intramuscular or intravenous injection of 10 grains of quinine bihydrochloride without delay. The British might get hyperpyrexia from purely physiological causes, but, of course, any added infection increased the liability very much. Focal sepsis often plays a very important part. It should always be remembered in the case of people going out to the tropics, that if they have septic teeth, septic tonsils, or anything of the kind, and then are exposed to this intense heat they are liable to get hyperpyrexia. It is very important to have a clean body as far as possible when going out to dangerously hot climates. One interesting point: when out in these climates and the tem-

perature rises to 110° F. in the shade, there is a certain amount of danger, when it reaches 115° F. you begin to get a number of cases of heat effects, and each degree rise above 115° F. means a very greatly increased rise in the incidence of cases of effect of heat. In 1917, the temperature rose to 122.5° F. in the shade. I would like to stress the autointoxication factor in the causation of these severe cases of the effects of heat, as it has an important bearing on the treatment. Normal saline should be freely given by the mouth and rectum, or subcutaneously or intravenously, also lumbar puncture is of advantage in cases of heat hyperpyrexia with cerebral symptoms. In addition to measures to reduce the body temperature by means of sprays of ice-cold water, fans to promote air circulation, everything should be done to promote excretion of toxins by the kidneys and bowels. In a large number of the more serious cases in Mesopotamia, heat hyperpyrexia comprised about 72 per cent., and there were two other types which were very curious. One I called the gastric: in this type of case, the patient who had been exposed to heat suffered from flushing of the face, nausea, vomiting and mental irritability and depression. These symptoms might continue for several days, the temperature being normal, and then suddenly hyperpyrexia would occur. When these symptoms appeared, it was necessary to admit the patient to hospital and adopt active treatment such as liberal liquids to drink, alkalies by mouth, colon irrigations and saline aperients, and so get rid of the toxins; 16 per cent. of the more severe cases were of the gastric type. Another type was the choleraic. Patients developed very acute diarrhoea and vomiting, almost like cholera, with a temperature of 102° F. or 103° F. They would then develop hyperpyrexia; 11 per cent. were of that kind. The milder type of case was "heat exhaustion," which occurred in great numbers if there was any movement of troops in the hot weather. Dr. LEE correctly said that the system which responded most to the effects of heat was the nervous system. I will conclude by describing a case I saw on Jubilee day, 6th May, 1935. This was a man of 80 who had on a tight-fitting court dress. He had been in the cathedral and then had to wait in the sun for 2 hours outside for his car to come. He sweated enormously. He had weakness in the legs, collapsed and was taken to the nearest hospital where a right hemiplegia was found to have occurred. Blood pressure was normal, heart normal, kidneys normal. This was really a cerebral vascular lesion (thrombosis) due to the effect of heat. I am glad to say that the symptoms have cleared up almost completely (25th May, 1935). One commonly sees nervous manifestations of various kinds in persons who have been exposed to extreme heat.

Major-General Sir John Megaw : I congratulate Dr. LEE on a very interesting and instructive paper. I agree with him that there is a need for greater attention to the physiological effects of tropical heat, but I think the study should be directed towards the prolonged and insidious effects of exposure

to tropical conditions, even more than to the dramatic effects of extreme heat. We have a satisfactory knowledge of the conditions in which acute illness results from exposure to heat ; we also know how to treat and prevent attacks of heat-stroke. Dr. LEE did not refer to two of the contributions to our knowledge of heat-stroke which impressed me more than any others : one of these is the very valuable analysis by our PRESIDENT of the climatic conditions prevailing at the times of occurrence of cases of heatstroke in India ; the other is by Professor HALDANE who has shown what happens when human beings are subjected experimentally to high temperatures either alone or in combination with high humidity and physical exertion. I hope Dr. LEE will be able to follow up his studies and investigate the deterioration which takes place in the body of residents in the tropics, and also the question of adaptation to high temperatures ; the latter may turn out to be analogous to adaptation to high altitudes, namely, adaptation up to a certain point followed shortly by diminished capacity for resistance to the unfavourable environment. Dr. LEE makes no distinction between heatstroke and sunstroke. I agree with him in this, but some experienced observers are quite convinced that sunstroke is a distinct entity. I hope those who hold this view will produce the evidence on which they base the distinction between the two conditions. The attitude of the " man in the street " towards any attempt at ameliorating the conditions of tropical life has been extraordinarily bigoted in the past ; 24 years ago, when I proposed to construct a cool room in the School of Tropical Medicine in Calcutta, the engineers objected on the ground that such a room could not be constructed. Then an able refrigeration expert, the late Mr. Wilcox, undertook to build a room to comply with my requirements and agreed to forego any payment if the result was not entirely satisfactory. The engineers fell back on the argument that the room would be dangerous to its occupants. The room was ready for use before the outbreak of war, but it was not actually occupied till 1920. My staff at first felt very doubtful as to the safety of the room, but after observing that it had no evil effects on myself they actually asked for another to be constructed. A few years later, the *Morning Post* discovered the existence of this room, and in an editorial dated 7th May, 1924, made a caustic commentary on the originators of the scheme. The following quotation shows the attitude of the *Morning Post*. . . . " But what would the men who fought at Marathon say about this central cooling ? What would the men who defeated swarthy hordes and crossed plains and rivers and scaled mountains and founded empires, and, having performed these mighty feats, quenched their thirst in brandy concoctions and foaming English beer, what would these men think of the highly-paid officials and business men who must have a temperature of 65° F. before they can undertake the business of the day ? Are even our proconsuls deteriorating ? Can England hold India only in a temperature of 65° F. ?

In the old days, it was the East that was accused of spoiling the West ; now it is the West that is spoiling the East."

I do not think Dr. LEE is likely to be deterred by public opinion from making efforts to improve the climatic environment of residents in the tropics, and I hope he will get on with the good work.

Dr. G. P. Crowden : I have really very little to say after what we have heard from the eminent authorities who have spoken this evening on the subject, but I should like briefly to indicate one or two points of view. First of all, just as in military matters so in disease, if we are faced with superior numbers we have to defeat them in detail. I think you will agree that Dr. LEE has faced us with superior numbers of minute physiological problems, all associated with the general problem of the effects of heat on the body. In studying a subject so complex from the point of view of intimate physiology, I feel sure that he will have to confine his studies to one or two aspects of the problem at a time, and so build up information which will enable him to construct a diagram or plan correlating the varied aspects of the problem and orientating them in relation to the body as a whole. Again, having regard to the disturbance to the subject, it is not impossible that if innumerable observations are made on one subject at one time, then his reactions may be influenced by experimental routine and differ in degree and possibly in kind from those of a normal individual under similar environmental conditions.

There is another aspect of the problem to which I should like to draw attention—namely, the variation in reaction to heat shown by different individuals. This fact was borne home to me by the data collected by Dr. DREOSTI, one of the medical officers to the Rand mines, who carried out a very extensive investigation on the reactions to work at high temperatures and high humidities shown by several thousand native applicants for employment. By observing body temperatures before, during, and after a given spell of work in a test chamber kept at 95° F. dry bulb and 90° F. wet bulb, he was able to group the men according to their reactions in respect of body temperature rise and pick out those who showed a predisposition to heat exhaustion or heat hyperpyrexia and who should be given acclimatisation work before being allowed to do full work in the mines. Their reaction to heat differed markedly in degree—a fact which would not have been apparent unless large numbers had been studied.

Dr. LEE referred to the alteration in endocrine balance as one of the chief factors in acclimatisation. Associating that suggestion with the action of the suprarenals in the case of cold, it seems possible that those glands may play some part. Adaptation in respect of capacity for sweating appears to be one of the main adaptations of the body to hot climatic conditions or work in hot environments.

In conclusion I should like to congratulate Dr. LEE on his enthusiasm in attacking this difficult problem.

Lt.-Col. W. F. Harvey : I did some experimentation in my early days, not so much on heat as productive of symptoms in man, but on its sterilising effect

at lower temperatures upon micro-organisms, particularly bacteria. In India the temperatures which may be obtained in the direct sun are of very considerable magnitude, much higher probably than people imagine. They may rise, as I have myself tested, to 70° C., which is a level of temperature certainly unfavourable to the lower organisms, in fact a sterilising one, and distinctly uncomfortable for man. It is possible, with time, to cook an egg in the sun. I have tested on one or two occasions the effect of heat on myself. One of the earliest was an involuntary test: It consisted of getting into a first class carriage in a train and travelling only a matter of thirty miles. At the end of it, I felt extraordinarily ill. When I returned at a later date to the station from which the train had started, and suggested to the station master that first class carriages should not be allowed to stand out in the sun before they were set up, he met me with the objection that it was very difficult to prevent this, and that he would like to know what was the remedy for reducing the temperature. I told him there was a very simple remedy, namely, to water the roof. He said he had tried various types of remedy, and would not have it that any was effective. The second occasion was an experiment on myself, on which I placed myself in an artificial enclosure, in that way avoiding to the fullest extent the effect of convection currents, and sat in the sun with a simple felt hat on my head. The altitude was 6,000 feet above sea level, but on that occasion I very rapidly became uncomfortable and had to give up the experiment in ten minutes. When I looked at the thermometer which I had placed inside my hat, it had reached 110° F., and where the hat had tipped down to touch my skin I had developed a definite burn. These experiments will show you the rate at which the direct sun's rays are capable of acting and are, I think, of some importance. In Mesopotamia there were many opportunities to study the effects of sun radiation. I have read of the development of a temperature in Muscat of 87° C., and in one of the R.A.M.C. reports on the conditions in Mesopotamia, it was stated that water tanks had been known to reach a temperature of 85° C. These, of course, are effects of direct sunlight. I would ask Dr. LEE—he emphasised particularly the effects of desiccation, dehydration and presumably anhydraemia—what would be the histological effects corresponding to these states? I have experimented on rats, which have no sweat glands except possibly in their paws. I subjected the rats to high temperature with high humidity, so as to bring the atmosphere surrounding these animals to saturation point and give no opportunity for evaporation from the skin surface. The experiment was devised to emphasize the importance of humidity in heatstroke. When these animals were brought to examination histologically, there were two lesions quite distinctive and worth following out, though I had not the opportunity to do so. There was a very marked cerebral oedema, and an extreme degree of pulmonary oedema; one got, instead of desiccation and dehydration, the reverse condition of water-logging.

Dr. Horace Smirk: I was very much interested in Dr. LEE's communication, and particularly appreciated his plea for the absence of any artificial simplification of such a complicated subject. I have no personal experience of tropical work, but happen to have worked on the effects of dehydration and of changing the electrolyte concentration of blood upon the urine flow. Dr. HELLER and I made experiments on rats and rabbits. We placed the animals in a chamber at 37° C. In this environment they breathed rapidly; there was considerable loss of water through the lungs, and because these animals have no sweat glands it was a loss of water and not of water plus salts. We left them in the hot chamber until they had lost 5 per cent. of their body weight of water and then removed them from the chamber and gave them back 5 per cent. of their body weight of water by means of a stomach tube. Each animal had therefore about the same amount of water in its body as when it started. It might be thought that there should be no diuresis, but actually an excellent water diuresis occurred in each animal. It is clear that in the rat and rabbit dehydration is not sufficient to prevent the development of water diuresis. We performed another experiment pointing in the same direction. Water was given to rats under normal conditions, and when the diuresis was established we put the rats into a warm chamber at 37° C. Before long the urine flow diminished and finally ceased. We then removed the rats from the chamber and found that the rate of urine flow began to rise again and the animal went into a state of diuresis. Under these conditions in the rat, the inhibition of the urine flow was not due merely to the state of dehydration, but to some other factor associated with a rise in external temperature. I do not suggest that these experiments can be applied directly to man, for man in a hot environment loses not only water in the sweat, but also salt. The observations, however, may indicate that we should try to decide whether in hot climates it is the dehydration (as such) that causes the diminished urine flow in man, for in the rat and rabbit simple dehydration is not the explanation.

Dr. LEE spoke of the effects of electrolyte imbalance. Dr. BALDES and I made some experiments on the effects of mineral-free and mineral-rich diets on man. First we placed normal men on a mineral-free diet and thus induced a considerable fall in the plasma chloride and total osmotic pressure of the plasma. Secondly we gave large doses of salt solution and found, as a result, a rise in the total osmotic pressure of the plasma. We observed that both in the salt-starved and in the salt-enriched subjects that the rate of urine flow, after giving say a litre of water, was very much less than the normal. The results we obtained could not be due to depletion of the body reserves of water for in some experiments we did not give merely one litre of water but three successive litres of water, and yet the rate of urine flow was less than that obtained from one litre of water in the normal subject. It seems, on an experimental basis, that either a rise or a fall of the electrolyte concentration of the blood tends to reduce the response of the kidneys to water administration, and to conserve the water reserves.

Lt.-Col. H. H. King : 'There are four clinical conditions mentioned in the paper, and I suggest there ought to be a fifth, not hyperpyrexia but *pyrexia*. Once after a whole day in the sun on a river bank in Mesopotamia I found I had, by the evening, a temperature and headache ; and for 3 weeks I had an extraordinary temperature unlike that of any known illness—I was not very ill and had a clean tongue. I was a prisoner at the time without any opportunity for laboratory tests, but as far as I could judge there was no definite disease such as paratyphoid, and I put my symptoms down to damage to the temperature-regulating mechanism. If damage of that kind is possible, and if it can cause fever for one, or two or three weeks, then it is a clinical entity and should be recognized as such.

A second point is that if the temperature of the body is raised, as by being in a steamy atmosphere, the results seem to be different from those in dry countries like Mesopotamia where there appears to be, in addition to the general effect of the heat, a direct local action of radiant sunlight on the brain. I was once travelling in East Africa in a goods wagon, practically shut up and in the dark, but with enough fresh air for ventilation. I was surprised to find myself (though with no apparent rise in my temperature) getting a headache from the heat radiated from the hot, dark sides of the iron railway wagon. To be more comfortable I had to put on my pith hat. I think those who recognize a difference between sunstroke and heatstroke are correct ; and that radiant energy does local damage. If lumbar puncture is useful in the treatment of sunstroke, this also tends to prove the existence of local damage.

I raise these two points : Whether there is not a fever solely due to damage by heat to the temperature-regulating mechanism ; and whether there is not a local damage by radiant energy in the absence of a primary raising of the body temperature.

The President (Sir Leonard Rogers) : Some 30 years ago I was led to study the conditions influencing the occurrence of heatstroke in India by the publication of a theoretical paper by a home writer attributing the condition to a microbial infection on the strength of assertions quite contrary to experience in hot climates. Thanks to the kindness of R.A.M.C. officers, I obtained the dates and places of 424 cases of heatstroke in the British Army in India in 3 years, and also the meteorological records on the places, and at the time of their occurrence, and these disproved every statement on which the microbe theorist had based his hypothesis. Briefly, it appeared that with a relative humidity well below 60 per cent., heatstroke cases became very prevalent with a mean temperature of 108° F. and a maximum one of about 118° F., but in such climates as that of Bengal, with a relative humidity of over 60 per cent., cases commonly occurred with a mean temperature of 98° F. and a maximum one of about 108° F., owing to the cooling effects of the evaporation of insensible perspiration being largely lost with such a humidity. I also studied cases of heatstroke for a number

of years in the Calcutta European Hospital, and found that even with a temperature up to 109° F., recovery took place if the patient had not been unconscious for more than $1\frac{1}{2}$ hours, but not after 3 hours. Cold pack under a punka, and cold water rectal enemas, reduced the temperature best. After finding that hyperpyrexia after hypertonic saline infusions in cholera were prevented by injecting the fluid intravenously at a temperature of about 80° F., I arranged to try injecting one pint in 5 minutes of normal saline at 60° F. into the internal saphenous vein at the ankle to cool the blood as rapidly as possible; for I had noted dehydration as shown by red corpuscle counts of 6 to 7 million, and I had cold saline kept ready, but although I frequently cycled to the hospital on the afternoons of heatstroke weather, I never obtained a suitable case for the test. I tried gum solutions in cholera, but found them deadly and doubt their advisability in heatstroke, but a slightly hypertonic and alkaline infusion might be of use in some cases. LONGMORE about a century ago pointed out that frequent micturition was a premonitory symptom of heatstroke, possibly due to the concentrated urine being irritant.

Dr. D. H. K. Lee (in reply): When I came here to-night, I felt like a very small Daniel in a den of hungry lions, but, whatever the cause, the lions have dealt very leniently with me, and I must thank them for their forbearance. It is quite impossible to deal here with all the points raised *seriatim*. CILENTO, in the introduction to *The White Man in the Tropics* complains of the lack of cohesion in the existing body of physiological and biochemical knowledge concerning the effects of tropical climates. This complaint has some justification, and for that reason I have preferred to give an account of general principles rather than to detail personal experiments.

Acclimatisation was mentioned by several speakers. This was the question which first attracted me to this field, but it is one which must be attacked by logical steps. The first is to know what are the effects of heat upon unacclimatised persons; this is the initial fundamental knowledge required. The immediately succeeding steps would be to know what changes occur in these reactions during the process of acclimatisation, and thirdly, how fully acclimatised people react to the same circumstances of external heat. My own conscious experiments have been directed towards a determination of the first group of data, from which I may proceed with more confidence to the second and third steps of the problem. Anything I can say at present concerning acclimatisation would either be already known to you or would be a sheer guess. On general grounds, as I have indicated, the endocrine glands and the circulatory system might be expected to provide convenient mechanisms for this purpose. The importance of psychological adaptation should not be overlooked.

Dr. CROWDEN mentioned the probable importance of sweating as a factor in acclimatisation. While one might expect this to be so from general considerations, one finds the most contradictory statements in the literature.

In the face of this, one can only re-investigate the matter for oneself. So far I have no definite evidence to submit.

I very much welcome the criticisms brought up by various speakers, since I want to know what people actively engaged in the practice of medicine in the tropics think about these problems. I am particularly anxious to elicit their observations, since such observations constitute valuable material. In this way the laboratory experiments can be made to traverse the paths most likely to be of value in tropical problems.

COMMUNICATIONS.

THE STRUCTURE AND DEVELOPMENT OF *PLASMODIUM FALCIPARUM* GAMETOCYTES IN THE INTERNAL ORGANS AND PERIPHERAL CIRCULATION.

BY

J. G. THOMSON, M.A., M.B., CH B., M.R.C.P.

AND

ANDREW ROBERTSON, M.B., CH.B.

Factors Influencing Gametocyte Formation.

Since the discovery of the malarial parasite of man in 1880 by LAVERAN, who was the first to observe the phenomenon of ex-flagellation of the male gametocytes in drawn blood, the literature regarding the development of the sexual elements is by no means extensive. Indeed the stimulus, which results in the production of gametocytes, and the factors influencing this production are still largely matters of speculation not yet capable of definite solution although various attempts have been made to correlate the appearance of gametocytes with certain stages of the asexual cycle or with certain periods of the infection as a whole. It has been suggested that particular sporozoites inoculated by the mosquito are from the beginning predestined for the production of gametocytes but general opinion tends to discount this theory and rather to favour the idea that the crescents originate from merozoites following the action of an unknown stimulus. Such a stimulus might be provided (THOMSON, D., 1914) by the gradual or increasing development of some bodily resistance or immunity to the continuance of the asexual cycle, or conversely, as was held by SINTON, BAILY and CHAND (1926) by a lowering of this immunity, a condition which might possibly be brought about by an alteration in the pH of the spleen. In the same way THOMSON, D. (1914) held the view that insufficient dosage of quinine seemed to favour the output of crescents and GREEN (1929) came to the conclusion that administration of quinine tended to increase crescent formation. These contradictory views may be reconciled if it is borne in mind that an apparent increase in the formation of crescents, as was pointed out by

GARNHAM (1931), may be due to the ordinary wave of crescent production and that the occurrence of this wave cannot definitely be attributed to quinine administration or the lack thereof. This same worker (GARNHAM) failed to stimulate crescent formation by injecting serum from cases with large numbers of crescents in the blood into patients freshly infected with the disease. Experimental evidence, therefore, is lacking which would associate the wave of crescent increase—and decrease—with any reaction of the host to the parasite.

Seasonal variations have been noted in the appearance of crescents in the peripheral circulation (SINTON, BAILY and CHAND) but it seems probable that these seasonal changes should be interpreted (GARNHAM) not as being peculiar to the crescents but rather as an indication of the existence of different clinical types of the disease. It would appear to be an accepted fact that fresh infections and what may be termed acute relapses tend to produce crescents in large numbers whereas the more chronic or latent forms, perhaps as a consequence of the paucity of asexual parasites, show few gametocytes. Epidemics of malaria and the usual rise in localities exhibiting a definite seasonal incidence of malaria will be followed by a large number of crescent cases, and this will be repeated during the period when relapses may be expected. The presence of gametocytes depends on acute infections and they are most numerous when malarial transmission is at its maximum.

GARNHAM (1931) drew attention to the fact that the crescent rates in the indigenous population of tropical Africa may, at first sight, appear to be extremely low, for out of about 4,000 persons in Kenya Colony examined for malaria by the thick film method, only 2 per cent. showed crescents whereas the parasite rate, according to age, environment and other factors, varied from 50 to 100 per cent. If continued observations on each case are substituted for the method of random sampling it is found that, sooner or later in the course of the disease, nearly 100 per cent. will show crescents. Out of 183 cases examined in this manner by GARNHAM, 87 per cent. eventually produced crescents while the cases in which they were not detected were those of early death, early discharge from hospital, extreme scantiness of the asexual forms, or, in a few instances, cases in which no particular cause could be assigned. Further emphasis has been laid by THOMSON, J. G. (1935) on the necessity for frequent examinations of each case, as distinct from the method of random sampling, for in carrying out a survey on 103 native children, living in Nyasaland under the same hyper-endemic conditions, once a month throughout an entire year it was shown that the number of occasions on which gametocytes occurred were *P. falciparum*, 47; *P. malariae*, 47; and *P. vivax*, 9. This is in marked contrast to the monthly returns; during the month of April, when malaria is most prevalent, crescents were found in only four of the children. This survey also showed clearly that the young children up to 2 years of age who suffer from acute infestations, always harboured most gametocytes and that thereafter the number of gametocytes progressively diminished as age advanced. Age, *per se*, does not appear

to have a bearing on the formation of crescents except in so far as immunity to the infection as a whole increases with increasing age.

The Curve of Crescent Formation.

Reference has been made above to the fact that crescents tend to appear in the peripheral blood in the form of a wave. Attention was first drawn to this by THOMSON, D. (1911) who, by means of a series of daily enumerations, showed that the crescent curve tends to follow a fairly constant course. The first appearance of the crescents occurred about the 10th day after asexual forms could be detected in the blood stream. In this connection SINTON has noted the appearance of the crescents in from 5 to 10 days while GARNHAM found that the average period elapsing between the onset of symptoms and the appearance of crescents was 11 days. Thereafter, when the original attack was confined to a single isolated paroxysm, THOMSON was able to demonstrate that the crescent curve rises to a peak and then rapidly falls again, thus indicating that the majority of crescents must perish in a few days time. Admittedly, in the absence of quinine administration, a single paroxysm of this kind is a rarity and, when the clinical aspect of the disease follows more usual lines, the crescent curve will be quite different from the foregoing, tending to be prolonged and to form a plateau with several sharp subsidiary peaks. The plateau may be observed in cases in which, even if no quinine is given, there is no fever to indicate the presence of the asexual cycle. This may be explained on the grounds that the asexual forms, though few in number, continue to produce new crescents.

The length of time during which the individual crescents may persist in the peripheral blood, in other words the life of the crescent, has a very definite bearing on the outline of the crescent curve. The evidence available tends to show that the development from the ring form takes about 10 days and the life of the crescent in the blood stream is restricted; as a rule crescents disappear from the blood stream in about 15 days from the start of quinine treatment. The occurrence of crescents in the peripheral blood for prolonged periods—e.g. 8 weeks, THOMSON, D. (1911); 60 days, SINTON (1926); or continuously for as long as 128 days, GARNHAM (1931)—obviously is due not to the persistence of the individual crescents but to their continued formation, even in instances such as that quoted by GARNHAM where the malaria was complicated by osteomyelitis of the tibia, in which there are no obvious febrile relapses and in which large doses of quinine had been administered. From the work of JAMES (1932) it is now a recognised fact that certain strains of *P. falciparum* are resistant to quinine and it is quite probable that these resistant strains might continue to produce crescents over a prolonged period. This fact, therefore, that the life of the crescent is limited, indicates that for the maintenance of the crescent curve in the form of a plateau, continued production of crescents is necessary. It should be noted, however, that GARNHAM found, in spite of continued pyrexia, the

crescent curve tended to rise to a peak and fall sharply and did not produce the plateau described by THOMSON.

The Development of the Crescent.

MALCOLM WATSON (1903) appears to have been one of the earliest observers to describe the developmental stages of crescents. This worker published illustrations of oval and spindle-shaped forms in the peripheral blood of a case with a severe infection from the Federated Malay States, and also noted the

DESCRIPTION OF PLATE.

(Figs. 1 to 26 were drawn at a magnification of $\times 2,600$ approx., Figs. 27 to 34 at a magnification of $\times 3,200$ approx.)

Figs. 1 to 31 were from the peripheral blood, while Figs. 32 to 34 were from sections of the spleen.)

Figs. 1 to 4.—Early stages in the development of the crescent. The forms at first are rounded, become ovoid and then spindle-shaped. Fig. 4 shows the decolouration of the containing corpuscle with the persistence of a pink staining band of haemoglobin along the margin of the parasite.

Figs. 5 and 6.—Developing crescents showing a thickening of the cytoplasm along the margins suggestive of the formation of a periplast or capsule. These forms frequently have one margin comparatively straight while the other tends to be curved.

Figs. 7, 8 and 9.—A further stage in the development in which the parasites are spindle-shaped.

Figs. 10, 11 and 12.—Spindle-shaped forms which exhibit the sharply tapered ends sometimes deeply stained characteristic of the developing female gametocyte.

FIG. 13.—Shorter, more rounded male crescent.

FIG. 14.—Two spindle-shaped gametocytes in the same corpuscle. These forms have taken on the deep purplish red colour which results from intense staining.

FIG. 15.—Female crescent showing remains of the containing corpuscle.

FIG. 16.—A short stumpy form such as occurs in the peripheral blood. These forms may occur in different parts of the world but seem to be especially numerous in certain parts of Africa.

Figs. 17 to 20.—Irregularly shaped developing crescents with the corpuscular inclusions described by GANNHAM (1931).

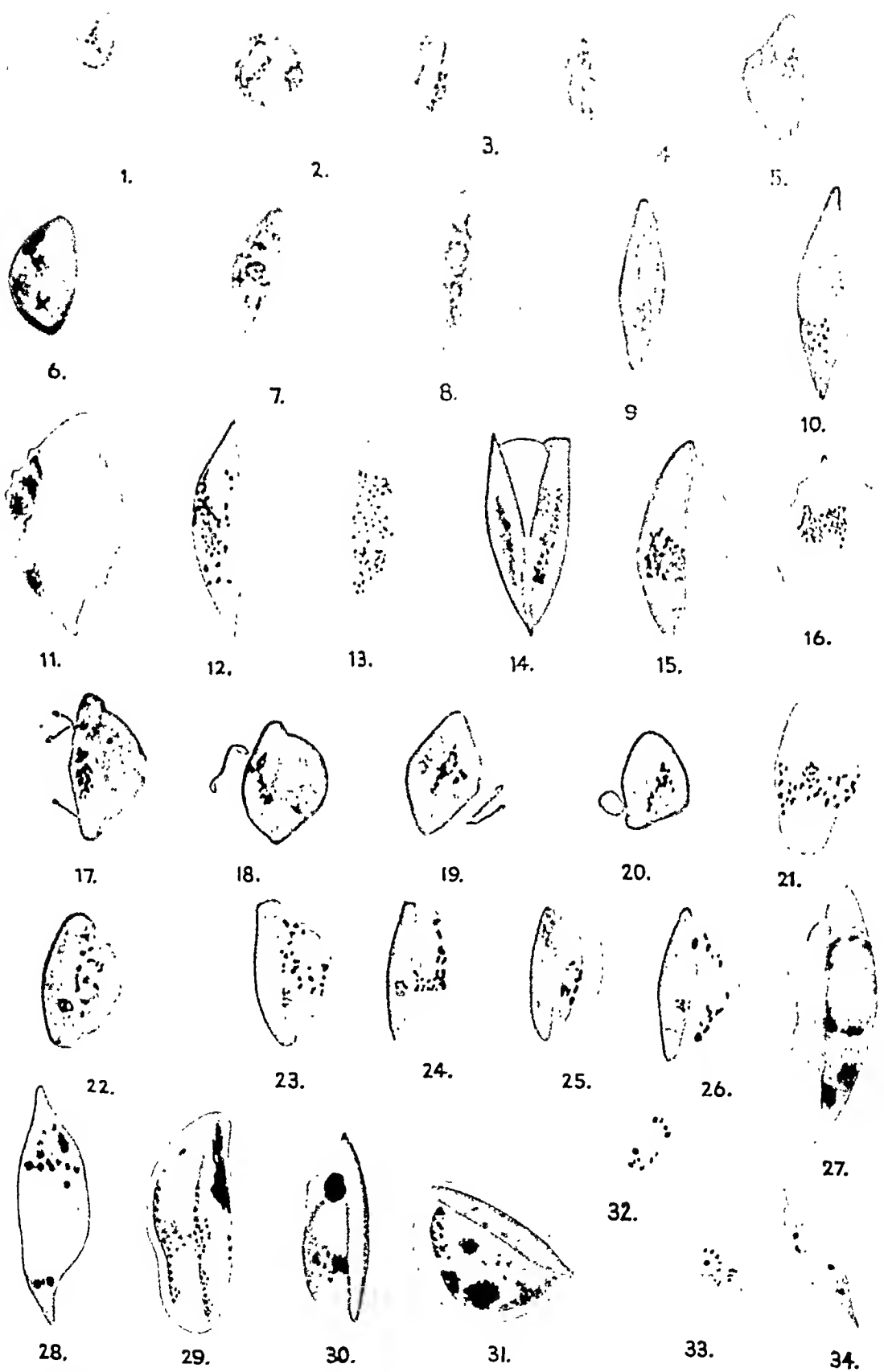
Figs. 21 to 26.—Spindle-shaped forms which have ruptured allowing the endoplasmic contents to extrude like a hernia.

Figs. 27 and 28.—Elongated forms showing the sharp pointed extremities and the displacement of the nucleus towards the end. They also illustrate the tendency of the pigment partly to be associated with the nucleus and partly arranged around the endoplasmic zone.

Figs. 29 to 31.—Ruptured spindle-shaped forms in which the extruded contents have left the capsule clearly defined. In one (Fig. 29) the chromatin has been retained within the capsule.

Figs. 32 and 33.—Developing gametocytes at a fairly early stage from sections of the spleen showing the vesicular character of the nucleus, the arrangement of the pigment around the nucleus and the scattered pigment in the cytoplasm. The pigment was partly removed before staining to allow the nucleus to be more apparent. These forms exhibit the tendency for one margin to be straight (*cf.* Figs. 5 and 6). The smaller size is the result of shrinkage due to the method of fixation.

FIG. 34.—A slightly more mature spindle-shaped female gametocyte from a section of the spleen showing the sharply tapering ends and the vesicular type of nucleus.





scattered pigment and pale staining protoplasm in the developing gametocytes. It was not possible to obtain ex-flagellation of these forms probably because of their immaturity. Next SWELLENGREBEL (1913) again depicted developing crescents which were spindle shaped and also these curious developing forms to which attention has been drawn by ARAGÃO (1930) and by GARNHAM (1931) and are also shown here in Plate Figs. 5 and 6. Typical elongated, spindle-shaped crescents were described by IOFF (1930) and they have also been illustrated by WENYON (1926) and by THOMSON and ROBERTSON (1929). These spindle-shaped crescents are not of common occurrence in the peripheral blood but they do appear from time to time in small numbers.

The development of the crescent in the spleen and bone marrow was first studied—in smears made postmortem—by THOMSON, D. (1914) in Panama, and it was shown that they originated from the ordinary ring forms. Even at a fairly early stage it was possible to differentiate the developing crescents from the asexual forms, for, after the disappearance of the vacuole from the ring, the cytoplasm of the former stained a faint greyish or yellowish blue in sharp contrast to the much deeper and intenser blue of the latter. The youngest stages tended to be spherical, some 2μ to 3μ in diameter, while pigment appeared fairly early and remained scattered throughout the cytoplasm. It is interesting to note that THOMSON did not describe the characteristic spindle-shaped crescents or the other curious forms seen by WATSON (1903), SWELLENGREBEL (1913), IOFF (1930), ARAGÃO (1930) or GARNHAM (1931). This might be due to the fact that his observations were carried out on postmortem material or again it is possible that races or strains of *P. falciparum* may vary morphologically in their developmental stages.

ARAGÃO (1930), working in South America, investigated the development of crescents during the life of the patients by utilising the technique of spleen puncture. His observations were important in that he was inclined to the view that from the earliest stages differences could be detected between the developmental forms of male and female crescents. He was in agreement with the description given by THOMSON, D., of the stage, immediately after the ring, in which the vacuole is lost and the shape is spherical. This form increases in size up to 4μ or 5μ in diameter after which the organisms become ovoid or elliptic and progressively more elongated until finally they reach the mature crescent form. These rounded forms, in ARAGÃO's opinion, are the precursors of the male gametocytes. The females, on the other hand, at their earliest stages are stretched across the corpuscle, almost filamentous, in a band form reminiscent of a quartan trophozoite. As growth proceeds, pigment, at first absent, appears as fine scattered granules and the parasite becomes broader with a tendency to having one border relatively straight and the other convex (*c.f.* Figs. 5 and 6). The extremities become tapered while the length increases up to 13 to 15μ and the spindle-shaped outline is still retained. Finally, the mature crescent— 12 to 14μ in length—is formed (Fig. 15). That there exists

an element of doubt regarding ARAGÃO's interpretation of these thin, band-shaped forms as the forerunners of the female crescents is evident since GARNHAM (1930), who also has studied material obtained by spleen puncture, stated that such forms were very rarely seen by him. With reference to the question of pigment, however, GARNHAM thinks that this may have a bearing on the differentiation of sex in the young stages for it "is always present, either a few small pieces at various points in the ectoplasm or as fine specks at the opposite pole to the chromatin; the former denotes the female gametocyte, the latter the male."

Further Observations on the Development of Crescents.

A series of blood films from Borneo, South India, Malaya, Kenya Colony and the Gold Coast have recently supplied abundant material for the study of developmental forms at all stages and, in addition, from two of the cases (Malaya) spleen smears, brain smears and sections of the spleen were also available for contrast with the forms, which were especially numerous, found in films of the peripheral blood before death.

With regard to the earliest stages, which are illustrated in the present instance (Figs. 1 to 4) from a smear of the peripheral blood of a native of Kumasi, West Coast of Africa, there is little to be added to the accurate description given by THOMSON, D. (1914). The smallest forms (Fig. 1), 2 to 3μ in diameter are round or slightly ovoid and tend to retain this outline until they reach a diameter of 4 to 5μ (Fig. 2). Pigment is formed early (Fig. 1), about the time when the vacuole disappears from the ring and it remains scattered, without any tendency to aggregation or localisation (Figs. 1 to 4), until the later stages when the crescent is reaching maturity. This arrangement of the pigment is quite distinct from that in the developing schizonts in which it is quickly collected into a round compact mass. The protoplasm of the developing crescents stains a faint greyish blue colour and the chromatin is more faintly stained than in the schizonts which show a much more decided blue colour of the cytoplasm. During these developmental phases very little alteration is found in the containing corpuscle or in its staining reactions.

There next ensues a gradual lengthening of the body with the formation of small oat-shaped structures tapering towards the ends (Figs. 3 and 4) and measuring 5 to 8μ in length by 2 to 3.5μ in breadth. The corpuscle, as a rule showing no signs of distortion in shape, often seems to be decolourised (Fig. 4) leaving a narrow strip of haemoglobin along one side of the parasite. At about this point forms begin to appear which are about the same length as the diameter of the corpuscle and which tend to have one margin comparatively straight while the other is markedly convex (Figs. 5 and 6). When intensely stained these forms show a deeply staining zone, purplish or red in colour, of peripheral ectoplasm about 0.5μ thick most frequently seen to best advantage along the straight margin. This deeply staining marginal zone is definitely a part of the cytoplasm of the parasite and is quite separate from the corpuscle; this fact

has already been noted by GARNHAM (1930). Such an appearance as this is strongly suggestive of the formation of a capsule or periplast and in any event, at this stage at least, it provides evidence that the cytoplasm is not homogeneous but has an outer, deeper staining and an inner more faintly coloured zone.

As growth proceeds the gametocytes not infrequently assume a diamond-shaped outline (Fig. 19) and during this part of the development the corpuscular inclusions described by GARNHAM (1930) are fairly common (Figs. 17 to 20). What the source of these corpuscular inclusions may be is a matter of doubt. Sometimes they assume shapes and adopt positions within the corpuscles which give reason to believe that they may be derived—perhaps by tearing or other accidental mechanism—from the periplast or outer layer of the developing crescent (Fig. 17); at other times they may lie within the corpuscle clear of the parasites and from their outline and colouration give a decided impression of originating from the corpuscle itself and bearing a close resemblance to Cabot's rings (Figs. 18 and 19). With increasing size and approaching maturity the cytoplasmic differentiation into a dense outer and an inner zone tends to become progressively more difficult to demonstrate, but that a fine pellicle or periplast does persist is obvious in films, where, in the act of smearing, rupture has taken place and the more fluid contents have partly escaped (Figs. 21 to 27, 29 to 31).

The tendency towards a spindle-shaped or fusiform outline (Figs. 7 to 9) becomes more definite while the ends are tapering with sharp, rigid points (Figs. 10 to 13) more noticeable in the female (Figs. 10 and 11) than in the shorter, broader, more rounded male (Fig. 13). While the present findings are not in conformity with the suggestion made by ARAGÃO (*vide supra*) that the female originates from thin band forms stretched across the corpuscle, nevertheless he was correct in his observation that the female takes a much more definite spindle-shape with more sharply defined tapering ends. These ends, too, are often densely staining (Fig. 10) while the endoplasm in the centre is much lighter. As the spindle elongates it may become curved and it should be noticed that, under these circumstances, the convex border is the one which originally had the straighter outline (Figs. 5 and 6), while the concave margin is the one which previously was the more convex. This bending of the straight margin may be the result of a slight pull or tension supplied by the remains or the envelope of corpuscle which is often seen stretched across the concavity (Fig. 11). The presence of two developing crescents in a single corpuscle (Fig. 14), as was pointed out both by ARAGÃO and by GARNHAM, is of quite common occurrence, and, like the other spindle-shaped crescents, if intensely stained with Leishman or Giemsa stain, they acquire a deep purplish pink tint which seems to be characteristic of these forms (Figs. 22 to 26). When fully developed (Fig. 15) the female crescent is deep blue in colour, the extremities become blunter, though still slightly more tapered than those of the male (Fig. 13) but the periplast cannot be detected possibly because it is thinner at this stage.

nearly always a certain amount lying, as it were, free within the endoplasm (Figs. 32 to 34), and, as maturity approaches, this sometimes becomes more localised at the junction of the ecto- and endo-plasmic zones (Figs. 10, 27 and 28). When rupture occurs during the act of smearing, the nucleus and pigment may be retained within the capsule (Fig. 29) or both may be included in the mass of extruded endoplasm (Figs. 30 and 31). The differentiation of endo- and ectoplasm is not so clearly defined in the male crescent, which as a whole tends to be lighter and more homogeneous in its staining than the corresponding female. Wet fixation and staining proves that the male does possess a large ovoid area of endoplasm within which are situated four to eight distinct nuclei (THOMSON, 1932).

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THE CLINICAL TESTING OF MALARIAL REMEDIES.

BY

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Experimental and clinical researches on the treatment of malaria have both shown that none of the usual remedies has an invariable action on malaria parasites. In actual practice each remedy must be used with due regard to its special properties, the point of attack of the remedy being the decisive factor. A remedy may be used against various kinds and forms of malaria parasites with a different action in each case. Therefore, in choosing a line of treatment, not only must the kind of parasite concerned be taken into consideration but also the form (asexual, sexual, sporozoite) which it is desired to influence. Further, the virulence of the parasite itself plays a part which varies according to locality, to the clinical stage of the disease (fresh infection, treated or untreated relapse), and finally to the capability of the host of withstanding the infection (racial differences, increased resistance due to repeated infection). Consequently these various factors determine the scope of use of each drug in treatment, sanitation and prophylaxis. In 1930 when I was given the task of carrying out the first clinical tests on atabrin I endeavoured to take these into consideration as far as possible (PETER, 1932), and the present paper aims at showing how far these preliminary clinical tests on a few patients provided correct data regarding the scope of action of this drug which has since then been tested under the most varied conditions.

Since our knowledge of the connection existing between chemical constitution and chemotherapeutic action leaves much to be desired, preliminary tests on animals are always necessary. Malarial infection in birds offers an excellent parallel to human malaria and enables a selection to be made of the drugs which are suitable for testing on human beings. In recent years plasmodial diseases in monkeys have been successfully used for these tests. However, it must always be emphasized that animal and laboratory experiments are useful only as a means of orientation and that the last word lies in the field of clinical medicine.

The testing of malaria remedies on artificially infected paralytics goes a step further than laboratory experiments. This method allows much more scope to the experimenter and therefore permits of a much wider range of observation. Nevertheless, one cannot consider a natural infection and an artificial infection in a paralytic as equivalents. As these differences have already been referred to (SCHULEMANN, 1935) it is only necessary here to emphasize one point, namely, that in these experimental tests that condition found in

bacterial diseases which one terms "immunity," is completely neglected and must remain so. It is, nevertheless, generally recognized to-day that not only do residents in endemic areas show an increased resistance to the local plasmodial species but also that repeated attacks of malaria increase individual resistance to the disease. Under natural conditions it is possible to rely on this resistance when carrying out therapeutic treatment but this cannot be done in the case of artificial infection. In artificial infection injection of infected blood introduces parasites in all stages of development. Proliferation of the parasites takes place only through the merozoite form. This developmental cycle is fundamentally different from that following infection under natural conditions through injection of sporozoites by mosquitoes. Even where artificial infection is produced by mosquito bites it is impossible to do more than imitate the conditions of a primary infection occurring in small children or in adults living in non-endemic areas. The malariologist is, however, particularly interested in the therapy of malaria both in its endemic and epidemic forms.

Apart from this the malarial treatment of meta-syphilitics necessitates an antagonism between malaria and the syphilis. Therefore in all probability the course of a malaria infection in the meta-syphilitic is different from that of a natural infection. Furthermore, as it is undoubtedly not permissible to draw conclusions from therapeutic experiences gained in one malaria region with regard to the disease in another, it appears to be still less permissible to draw a parallel between artificial and natural infections: it would be a case of attempting a comparison between things which cannot be compared. A final conclusion with regard to any method of treatment can only be drawn from observations on naturally infected individuals in their natural surroundings.

This fact is in my opinion not sufficiently considered in the *Third Report of the Malaria Commission of the League of Nations* (1933). Though the report is intended to provide suggestions which the practitioner should follow, as the subtitle indicates, it deals almost entirely with "controlled experiments." The conclusions drawn go too far and unfortunately do not take into account the experience which has been gained in general practice. Since a criticism along the same lines has already been made on several occasions it will suffice to suggest here that it would be advantageous to make a similar compilation of the experiences gained in practice so that the practitioner may see what has actually been achieved with the various available medicaments under different local conditions.

The first clinical test of atebirin which I carried out in 1930 in a endemic area* on patients naturally infected with malaria were instituted with a view to answering the following questions:

1. Is atebirin in any measure effective against a natural malaria infection?
2. If so, against which type of infection is it effective?

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3. Which stages of the various malaria parasites are attacked by atebirin ?
4. What dose is required to produce a cure ?
5. To what extent is atebirin tolerated and what is its rate of absorption and excretion ?

For this purpose two patients with benign tertian malaria and two with subtertian malaria contracted naturally were chosen. Further, a third case of benign tertian malaria produced by inoculation (first passage) was selected. Since we were anxious to discover the behaviour of the parasites during and after treatment it was necessary to make blood films from the patients regularly every three hours in order to have as exact a picture as possible of the changes undergone by the parasites. To have as a control the normal course of the infection the three hourly blood films were commenced 48 hours before the treatment was instituted. The temperature was taken when the blood films were made, while further, the urine and faeces were examined daily. The haemoglobin was measured before, during and after the course in each case.

In studying the blood films an endeavour was made to gain some idea of the actual concentration of parasites in the blood, though it was realised from the start that the figures obtained could only be comparative. However, comparative counts were sufficient for the purpose, since it was desired primarily to ascertain the proportion between the various stages of the parasite. It was evident that the complete disappearance or the gradual diminution of the number of parasites in the blood would become clear at the same time.

It was decided to count the number of parasites in proportion to 500 leucocytes since this method seemed to be the only one practicable for the purpose. Under field conditions with a minimum of personnel it is absolutely impossible to work with special apparatus such as a counting chamber, special pipettes or a suspension of bird blood, when such a routine has to be kept up for days at three hourly intervals. On the other hand comparative counts against the leucocytes allows of the use of the routine blood films, taken in every malaria station, and permits moreover of the working up within a given time of the large amount of material available even in the case of a few patients. Absolute figures can, however, be obtained by this method if the leucocyte count is made at the same time.

The physiological and parasitological variability in the number of leucocytes is cited as a source of error in this method. This factor, however, takes a secondary place in determining the proportion of the various stages of the parasite and which forms, if any, are damaged by atebirin and to what extent. The actual proportion existing between the various stages does not change whether the leucocyte count is increased or decreased.

According to the literature (HÜLSE, 1917; KLIENEGER, 1918; PÖCH, 1903; RUBITSCHUNG, 1925; SCHILLING, 1924, 1929, 1932; WERNER, 1927) the behaviour of the leucocytes in malaria is as follows :

At the onset of a malaria attack there is an inconstant and transient

leucocytosis following which there occurs a leucopenia which reaches its lowest point at the height of the fever. With the fall of temperature the leucocytes increase again to normal. In the fever-free intervals the leucocyte counts are either normal or subnormal rising in the convalescent period to the neighbourhood of the normal. If these facts are kept in mind comparative counts on the basis of the leucocyte value will always give satisfactory results.

In the space of this paper it is impossible to give the tabular results of over 800 counts. Instead, two simple charts are included giving the results in one case of benign tertian and one case of subtertian malaria. The number of ring forms, asexual developmental forms and gametocytes is given in proportion to 500 leucocytes. The times at which the blood specimens were taken are recorded on the abscissa of the chart, and the number of parasites on the ordinate. In the upper portion of the diagrams (Charts I and II) the temperature will be found.

According to the results in benign tertian cases the answers to our questionnaire run as follows :

1. Atebrin is effective against natural malaria infection.
2. Atebrin acts against the parasites of benign tertian malaria.
3. The ring forms of *Plasmodium vivax* are the forms most quickly and successfully attacked. They are the first to disappear from the peripheral blood. The first dose of one tablet may have a certain provocative action in that more rings may be present in the peripheral blood after the administration than before. Whether this phenomenon is due to an increased multiplication or to an expulsion of the ring forms from the internal organs has not been determined. The other asexual developmental forms are also rapidly influenced, but not so rapidly as are the ring forms : they persist in the blood for a longer period.

The gametocytes of *P. vivax* resist longest. One obtains the impression that at the beginning of treatment they are either not influenced at all, or only to a slight extent ; in fact, after the first dose a distinct increase in the number of gametocytes in the peripheral blood is observable. Their destruction sets in later, and their presence in the peripheral blood can be demonstrated over a longer period than is the case with any of the other forms.

The fever attack following the commencement of treatment is markedly shortened while the clinical symptoms are diminished. Parasitologically the late appearance of the ring forms in the peripheral blood may be demonstrated, while there is a diminished increase in the other asexual developmental forms.

4. In the cases under observation a total dose of 1 gramme of atebrin, i.e. 0.1 gramme twice daily for 5 days, sufficed to bring about the above results.

The findings in cases of subtertian malaria were as follows :

1 and 2. Atebrin is effective also against the naturally acquired parasites of subtertian malaria.

3. In subtertian malaria, as in benign tertian, the asexual parasites are rapidly influenced. The first dose which is insufficient to destroy these parasites has a provocative action and leads to an increase in the number of ring forms

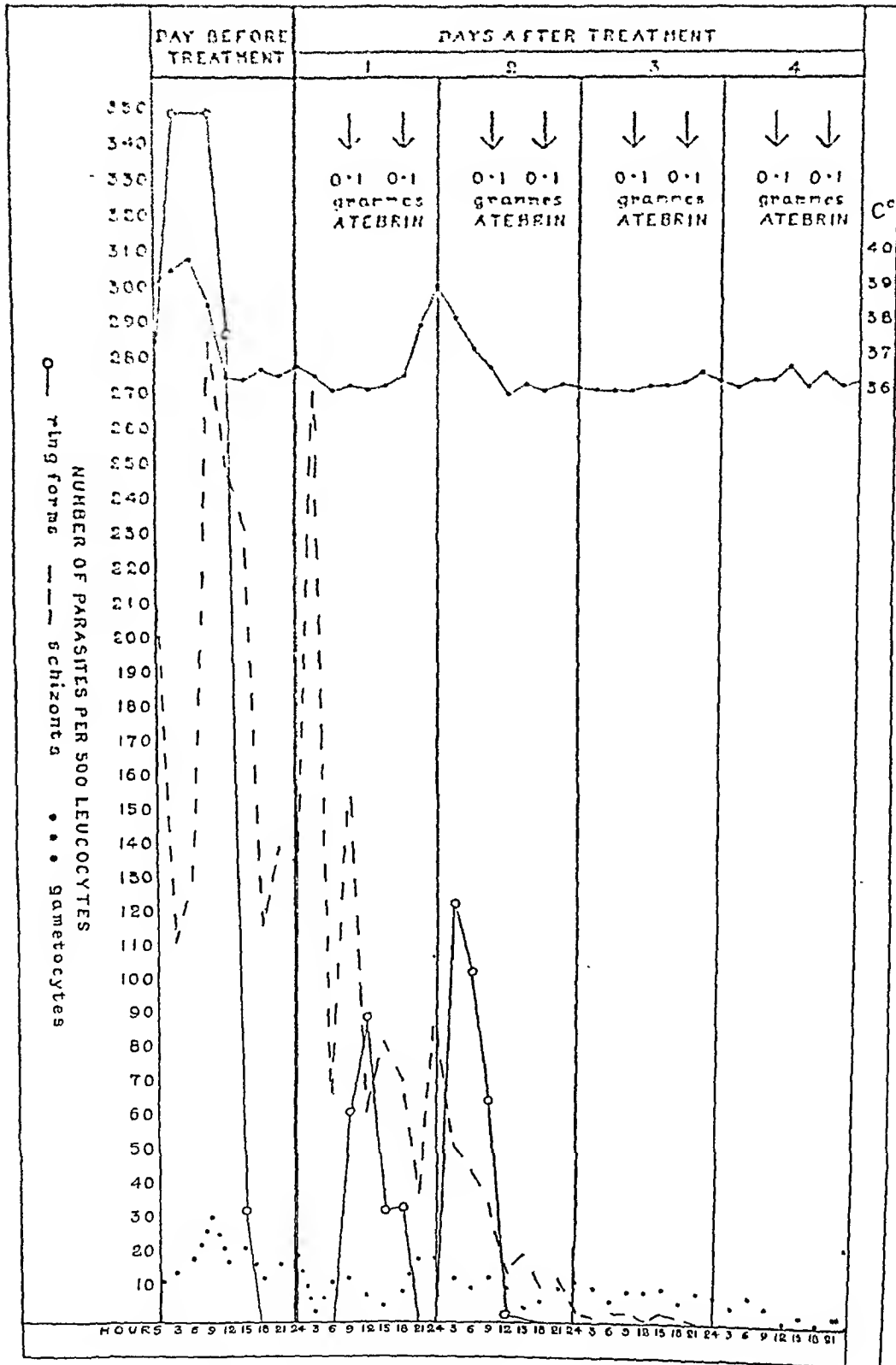


CHART I.—CASE OF BENIGN TERTIAN MALARIA.

in the peripheral blood. The subtertian gametocytes on the other hand are either not influenced at all, or only to a very slight extent. Even double the dose required to destroy the gametocytes of benign tertian malaria was administered without any marked effort. On the contrary the formation of subtertian gametocytes appeared to be stimulated by atebtrin administration. In the second case, for instance, observations made before treatment was commenced did not reveal the presence of subtertian gametocytes. These appeared however during the course of treatment, and after disappearance of the ring forms they were present in fairly large numbers.

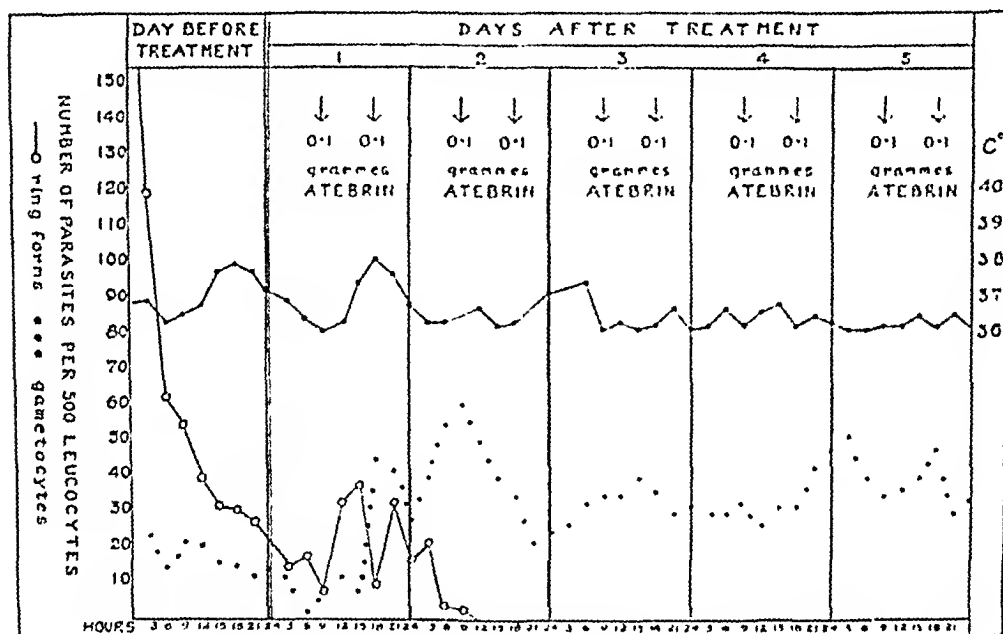


CHART II.—CASE OF SUBTERTIAN MALARIA.

4. To obtain the above results 2 grammes of atebtrin were given in the course of 9 to 10 days.

5. As regards the questions of tolerance of the drug and the rate of absorption, these preliminary experiments yielded a few important data. Atebtrin given in a daily dose of 0.2 to 0.3 gramme is well tolerated and gives rise to no unpleasant after effects. Excretion takes place through the urine and faeces. The drug can be demonstrated in the excreta for several days after its administration has been discontinued. In comparison with quinine and plasmoquine, excretion is slow. A yellowish tinge noted in the skin was, as the urinary findings showed, not due to icteric discoloration but to a deposition of the dyestuff under the skin.

The investigation has shown therefore that atebriu is to be regarded as a schizonticide both in benign tertian and subtertian malaria, the effect being produced in a very few days. In therapeutic doses it is well tolerated while it differs fundamentally from quinine and plasmoquine in its longer retention in the body.

Since April 1932, atebriu has been available for general use and over two hundred papers have been published on the results obtained during the last three years. Our experience has been confirmed and greatly amplified in different malaria regions and under the most varied conditions. As has been noted, differences can generally be accounted for by differences in local conditions. A few of these points will now be considered.

Since atebriu is an antischizont remedy its parasitological and clinical action in acute attacks is of particular interest. This is universally recognized. In this connection, while some authors report that the oral administration of atebriu gives results which are at least equal to, if not greater than, those given by quinine, others have found that its action begins more slowly.

Similar differences of opinion exist with regard to the dose and the duration of treatment. Although most authors agree that a 5 days course of 0.3 gramme daily is ample, references to treatment with daily doses of from 0.2 to 0.4 gramme of atebriu for 4 to 10 days are to be found.

In judging these differences of opinion the following consideration should be taken into account :—

Atebriu is a dyestuff, and its action on the parasites is in all probability a direct one. We have been able to demonstrate its presence both in the serum and in the red blood corpuscles by the fluorescent method. It would appear that the therapeutic effect is dependent upon the concentration of "atebriu" in the blood. Furthermore, atebriu has a marked affinity for the parasites to which it becomes very firmly bound (FISCHL & SINGER, 1934); on this account the amount required should be regulated by the number of parasites present rather than by the weight of the patient. Children would therefore require relatively higher doses than adults.

Because it behaves as a dye, tissues coming into contact with atebriu will be stained. Thus the buccal lining, oesophagus, stomach and intestine will take up the medicament to some extent so that at the beginning of a course, at least, a certain portion of the dose will not reach the blood with the consequent loss of the therapeutic effect of this amount. It will therefore be of advantage to give a higher dose at first in order to produce as rapidly as possible a high concentration of atebriu in the blood. A further argument in favour of this suggestion is the apparently provocative effect of insufficient preliminary doses.

In the treatment of malaria with atebriu it would therefore appear to be most important to obtain a high concentration of atebriu in the blood as early as possible. According to certain reports the parenteral route would seem to be particularly suitable for this purpose (ECKHARDT, 1933; HOOPS, 1933;

M. MAYER, 1933). A short time ago BLAZE & SIMEONS (1935) reported promising and rapid results with intramuscular injections of 0.3 gramme on each of two consecutive days. This fully confirms our theory. The explanations of the authors, however, as to the mode of action of atebtrin injections are not tenable.

It is clear also that metabolism must play a not unimportant part in the action of the drug and the following observation illustrates this fact :—

A patient having a *Plasmodium vivax* infection, the course of which appeared normal, received 0.3 gramme atebtrin daily for 5 days. The parasitological and clinical effect of the drug were both delayed, while on the other hand yellow staining of the skin appeared on the 3rd day. In less than 4 weeks parasitological and clinical relapse occurred. A second course of the same dose of atebtrin given under the same external conditions produced a very rapid clinical and parasitological cure without any appearance of skin coloration. During an observation period of several months no further relapse occurred. It would seem justifiable therefore to conclude that the metabolism of the drug can vary even in the same patient at different times.

It is not to be wondered at therefore that statements regarding the frequency and extent of the yellow staining of the skin should be contradictory. Thus in one region it is said to be very frequent while in another it is never observed. Marked staining indicates a deposition of atebtrin in the dermis. So long as it remains there the therapeutic effect of this quantity is lost. It is therefore understandable that where discoloration is marked the therapeutic effect is less. How far atebtrin which has been stored in the skin may still have a therapeutic action when released is as yet not known. It may be assumed, however, that at least a part of the atebtrin is broken up or changed into ineffective products. Even if we cannot explain why atebtrin is stored in the skin in one case and not in another, we can assume that, apart from individual factors (metabolism), racial factors and conditions of living (light irradiation), specially affecting individual malarial regions, influence the metabolism of the medicament in the body. In this connection I would refer to an observation made by JUNGE (1933). He gave relatively high daily doses of atebtrin prophylactically over a long period and noted a curious wavelike variation in the yellow colour although the actual daily dose remained constant.

Nutrition may also play a certain part in the therapeutic efficiency of atebtrin. As an extreme example of this, an observation made several years ago in the experimental laboratories of Elberfeld may be cited. It was noted that food-stuffs containing large quantities of cellulose absorbed atebtrin and prevented its absorption from the alimentary tract into the blood, thus diminishing its therapeutic effect. Large quantities of milk in the stomach are also said to hinder absorption.

Similar local and racial differences exist with regard to tolerance. In most reports it is stated that atebtrin given alone is well tolerated, on the other hand

several observers have stated that atebtrin causes gastric and abdominal pain. In two such cases under my observation I was able to establish the presence of a concomitant gastric ulcer. The acid content of the stomach is possibly connected with the degree of tolerance to the remedy. A point, however, which has been definitely established from various sources is that the simultaneous administration of plasmoquine with atebtrin diminishes the tolerance of the latter though here again marked local differences appear to exist. The reason for this has not been established, animal experiments having failed to provide any explanation (SCHULEMANN, 1935). In any case the main cause would appear to lie in the plasmoquine for when the dose of this drug is decreased from a tenth to a twentieth of the dose of atebtrin the tolerance is improved.

As regards relapses after atebtrin no data could be obtained from our preliminary experiments. However, the more extended tests which have been applied to the remedy through its use in practice have brought out a few facts even if the data cannot be analysed statistically because of the variability of the material. Thus it appears that the relapse rate in tertian malaria after the administration of atebtrin alone is considerably less than after quinine treatment. On the other hand it is higher than that following quinine plus plasmoquine, or atebtrin plus plasmoquine. The fact that JOHNSON (1934) found the unusually high relapse rate of 43 per cent. in Europeans in the same area in which he found only a percentage of 5 to 10 in Asiatics—an enormous difference—is to be attributed to differences in race, nutrition, mode of living, natural resistance to infection and medical supervision.

With regard to the relapse rate in subtertian malaria little material is available. Nevertheless, it appears that in this particular case atebtrin is more effective than quinine.

As with the treatment of the attack, so with the relapse rate, there exist great local differences which require careful examination and explanation in each case.

As regards individual prophylaxis atebtrin has always offered great possibilities on account of its slow elimination from the body. Experiments (JAMES, 1933; KIKUTH & GIAVANNOLA, 1933; SOESILO *et al.* 1933) have shown that atebtrin cannot be regarded as a causal prophylactic, that is to say it does not act on the sporozoites, but that unlike quinine and plasmoquine, atebtrin prolongs the incubation period. Several successful results have been reported. For instance SOESILO (1934) was able not only to protect children from clinical and parasitological attacks through the administration of regular doses of atebtrin, but also to free "carriers" of parasites. Experiments on a much larger scale are, however, necessary before any final conclusions can be drawn as to the value of atebtrin in prophylaxis. According to the results already obtained in adults daily doses of 0.2 gramme on 2 or 3 days of each week, or doses of 0.05 to 0.1 gramme daily will give an effective clinical prophylaxis. Professor SCHULEMANN and I have recommended these doses to the Malaria Commission of the League of Nations for experiments on a large scale.

It may not be out of place to conclude this discussion by quoting a sentence from an Editorial in the *Indian Medical Gazette* (1935): "atebrin . . . has been subjected to a very considerable amount of criticism during the last year and is on the whole coming out of it remarkably well."

SUMMARY.

1. The series of comparative parasitic counts carried out in the preliminary clinical experiments described in this paper have provided important data regarding the action of atebrin.

2. The conclusions regarding the mode of action of atebrin which were reached in this way have been confirmed during the subsequent three years of its use in practice.

3. Today it is justifiable to regard atebrin as an antischizont remedy, the value of which has been established in practice and with which a successful course of treatment can be carried out in a remarkably short space of time.

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ON FILTRATION OF MICROFILARIAE BY LYMPH NODES.

BY

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In 1933, one of us (C. K. D.) took part in series of experiments on the filtering efficiency of the popliteal and iliac lymph nodes in the dog. It was shown that when erythrocytes or streptococci, suspended in an artificial lymph, were perfused through these nodes at rates of flow and pressure consistent with normal conditions in the dog, a very complete filtration occurred. In the paper which reported these results* it was suggested that similar experiments utilizing microfilariae would be of decided interest.

Through the kindness of Dr. M. C. HALL, Chief, Zoological Division of the Bureau of Animal Industry, Washington, a dog was secured which was infected with *Dirofilaria immitis*. During the morning the blood of this animal

*DRINKER, C. K., FIELD, M. E., & WARD, H. K. (1934). The filtering capacity of the lymph nodes. *J. Experim. Med.*, lix, 393.

was heavily charged with microfilariae, and it proved easy to centrifuge the organisms out of the heparinized and haemolyzed blood. They were then resuspended in a solution of dog serum and physiological saline such that the protein content fell between 1.2 and 2.3 per cent., concentrations of the blood proteins normal for leg lymph in the dog. The microfilariae lived for many hours in such artificial lymph and were extremely active.

Just as in former experiments on filtration by lymph nodes, two types of experiment were performed.

In the first, an afferent lymphatic to the popliteal node was isolated and cannulated, the cannula pointing centrally. This cannula provided inflow of the lymph containing microfilariae. It is a fortunate fact that the afferent vessels to such a node all empty into the cortical sinus, and fluid introduced through a single vessel thus takes a path identical with that provided if all the afferent vessels are cannulated. Even more fortunate is the fact that the effluent from such nodes leaves ordinarily by a single short vessel which may be picked up at the hilus of the gland and in which it is easy to insert a relatively large cannula so as to avoid back pressure and distention. For convenience in perfusion, the inflow cannula was connected with a graduated reservoir in which necessary degrees of pressure could be maintained steadily and which permitted stirring of the perfusate so as to assure an even distribution of microfilariae. As soon as perfusion was started the total effluent lymph was collected in separate samples during short periods and all the microfilariae counted in each of these.

A typical experiment of the first type was as follows.

14th November, 1934. Weight of dog, 22.0 kg. Anaesthetized with 18 c.c. of 5 per cent. nembutal intraperitoneally. The afferent and efferent vessels of the left popliteal node were cannulated. Artificial lymph used for perfusion contained 1.37 per cent. dog serum proteins and 1,530 microfilariae per c.c. Table I presents the results.

TABLE I.

FILTRATION OF MICROFILARIAE. INFLOW THROUGH AN AFFERENT VESSEL OF THE POPLITEAL NODE. EFFLUENT FROM THE EFFERENT VESSEL OF THE NODE.

Time Min.	Perfusion Inflow.		Perfusion Outflow.		Total Number Microfilariae in Effluent.	Microfilariae per c.c. Effluent.	Perfusion Pressure mm. Hg.
	C.c.	C.c./Min.	C.c.	C.c./Min.			
0-10	1.45	0.145	1.6	0.16	0	0	20
10-25	1.70	0.11	1.2	0.08	1	0.8	15
25-40	1.35	0.09	1.32	0.32	12	9.1	27
40-55	2.95	0.19	2.70	0.18	53	19.3	27
55-70	1.55	0.10	1.60	0.10	33	20.6	28
70-85	1.55	0.10	1.60	0.10	26	16.2	20
Total	10.55		10.02				

As soon as all the perfusate had passed into the lymph node the animal was killed by bleeding to death and the perfused node removed promptly. It was cut in two parts, one

of which was fixed for sectioning. From the other, scrapings were made which showed active microfilariae with no tendency whatsoever to adhere to any of the cells scraped from the node.

The filtration accomplished in this and in duplicate experiments is in sharp contrast to what occurs when streptococci were perfused in a similar manner. These organisms rarely passed through the node, and on section were found adherent to reticular strands and in and upon phagocytic cells. The microfilariae meet no such biological restraint, and their imprisonment in a node is a simple expression of the mechanical complexity of the nodal filter.

In the second type of experiment, an afferent vessel to the popliteal node was cannulated to provide perfusion inflow. The effluent was secured by cannulating the thoracic duct at the entrance into the left subclavian vein. In these circumstances the artificial lymph containing microfilariae passed through two nodes, the popliteal and iliac, and entered the thoracic duct to be delivered with the normal lymph flow from the effluent cannula.

A typical experiment of this second variety was as follows.

27th November, 1934. Weight of dog, 16.3 kg. Anaesthetized with 12 c.c. of 5 per cent. nembutal intraperitoneally. An afferent vessel leading to the left popliteal node was cannulated and connected to the perfusion apparatus. The thoracic duct was isolated and cannulated. The artificial lymph for perfusion contained 1.8 per cent. dog serum proteins and 6,410 microfilariae per c.c. In order to indicate the time of arrival of the perfusate in the thoracic duct cannula, a small amount of 2 per cent. trypan blue in physiological saline was added to the perfusate. This dye has no observable effect on the activity of the microfilariae.

TABLE II.

FILTRATION OF MICROFILARIAE. INFLOW THROUGH AN AFFERENT VESSEL OF THE POPLITEAL NODE. EFFLUENT COLLECTED FROM THE THORACIC DUCT.

Time Min.	Perfusion Inflow.		Flow from Thoracic Duct.		Total Microfilariae in Sediment Thoracic Duct Lymph.	Microfilariae in Thoracic Duct Lymph per c.c.	Perfusion Pressure Mm. Hg.
	C.c.	C.c./Min.	C.c.	C.c./Min.			
0-12	1.7	0.14	13.7	1.14	0	0	20
12-25	1.3	0.10	13.7	1.05	15	1.1	20-35
25-41	1.4	0.087	13.5	0.84	8	0.05	20-30
41-56	2.4	0.16	13.3	0.88	110	8.3	25-30
56-71	2.2	0.14	13.0	0.92	102	6.8	25
71-86	2.35	0.16	13.2	0.88	197	14.9	25
86-99	0.95	0.073	13.3	1.02	457	35.1	20
99-114	1.00	0.066	12.3	0.80	511	41.5	20

Table II presents the results. In this case, the first specimen, which contained no microfilariae, showed no trypan blue. The dye appeared during collection of the second specimen. A small amount of heparin was added to each collecting tube to prevent coagulation of the lymph and the tubes were at once centrifuged at high speed. The counts of microfilariae represent all

the organisms obtained from the sediment thrown down in each tube. At the close of the perfusion the animal was bled to death and the popliteal and iliac nodes removed for scrapings and sectioning. Organisms recovered from nodes were normally active. Lymph taken by syringe from a large vessel just central to the iliac node contained 35 microfilariae per c.c., indicating the rapidity with which the organisms were passing through the nodes. The flow was somewhat higher than in preceding experiments but both grossly and microscopically the nodes were normal. The popliteal and iliac glands were thoroughly coloured with trypan blue, indicating passage of the perfusate through both structures.

HISTOLOGICAL EXAMINATION OF PERFUSED LYMPH NODES.

Sections 8μ thick showed small numbers of organisms when the entire cross-section of a perfused node was examined. The organisms were most frequently in intermediary sinuses in the depths of the gland, occasionally in cortical vessels, and rarely in the afferent vessels of the capsule. In no case was there the slightest evidence of reaction. The microfilariae lay free and apparently so far as the cells of the node were concerned were entirely without significance as foreign bodies.

In three experiments microfilariae were injected into nodes through the afferent vessel, and while perfusion was in progress the efferent vessel was tied so that the organisms were imprisoned in the glands. The microfilariae survived this treatment with great success. In the second experiment, the left popliteal node was injected with normally active organisms at 11.30 a.m., and the right node with organisms which were thought to have been killed by heat and which were non-motile when injected into the right popliteal node at 11.50 a.m. Both nodes were removed 22 hours later and scrapings from both showed normally active microfilariae. In the case of the right node, the contained organisms started their period of habitation in a somewhat precarious condition and their recovery is a good expression of the innocuousness of the lymph node environment. In the third experiment, living microfilariae were injected into a lymph node and imprisoned by ligation of the efferent vessels. Numerous microfilariae were found alive and normal at the end of 5 days. We cannot at present furnish any estimate as to the number that may have escaped from the node by migrating through the capsule or even possibly through the walls of large lymphatics. In none of these instances of imprisonment in nodes was there the slightest evidence of phagocytosis.

DISCUSSION.

These experiments show quite clearly that the microfilariae of *Dirofilaria immitis* pass through normal lymph nodes with great ease. They show further that the nodal environment for very considerable periods of time is in no way destructive.

The question at once arises as to how far these results with *Dirofilaria* can

be applied to human infection with *Wuchereria bancrofti*. *Dirofilaria* lacks a sheath and there is a rather general belief that unsheathed microfilariae are more motile in the sense of being able to travel from one point to another than are sheathed forms. In order to test this question of comparative motility, blood containing microfilariae was taken from our infected dog in heparin. The movements of single organisms were then charted, using a camera lucida and employing preparations both with and without cover glasses. These observations were made at room temperatures between 70 and 75° F. The results of such observations are shown in Figs. 1 and 2. In Fig. 1, pathways *A* and *B* have been

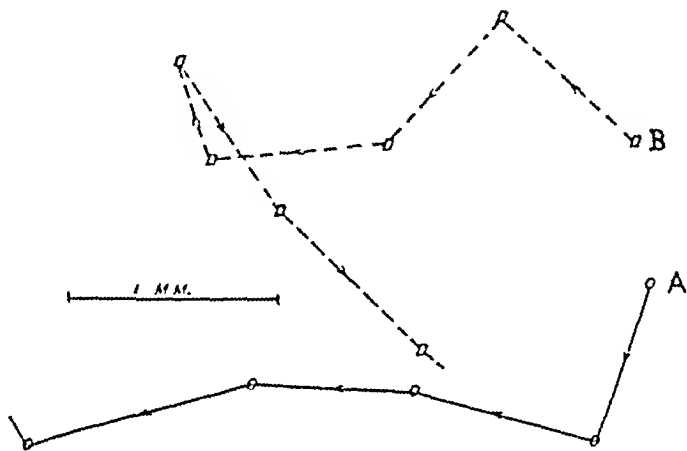


FIG. 1.—Courses of two microfilariae (*Dirofilaria immitis*) in undiluted, heparinized blood, plotted at 5-minute intervals. Observed in uncovered droplet.

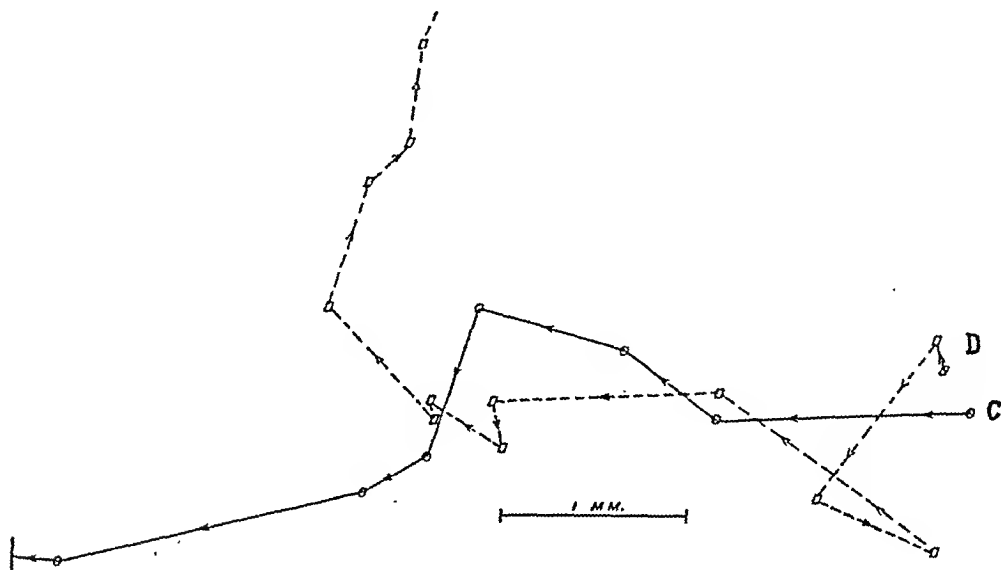


FIG. 2.—Courses of two microfilariae (*Dirofilaria immitis*) in undiluted, heparinized blood, plotted at 5-minute intervals. Observed under cover glass rimmed with vaseline.

travelled by microfilariae in blood without a cover glass. In Fig. 2, pathways *C* and *D* were made by organisms in blood and sealed under a cover glass by vaseline. When the four paths are measured and related to time the results are as follows : *A* travelled at an average of 0.18 mm. per minute ; *B*, 0.16 mm. ; *C*, 0.19 mm. ; and *D*, 0.14 mm. Unfortunately no infection with *Wuchereria* was available to us, but through the kindness of Dr. F. W. O'CONNOR we were able to obtain blood from a patient infected with *Loa loa* and thus could obtain comparative data upon a sheathed organism.

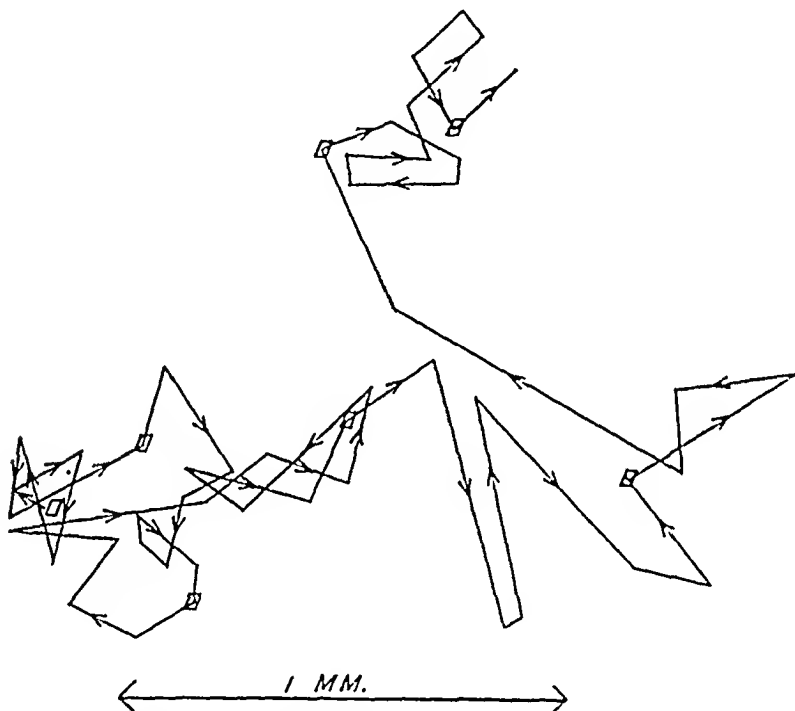


FIG. 3.—Course of microfilaria (*Loa loa*) in undiluted heparinized blood. at 5-minute intervals.

Fig. 3 is a chart of the movements of a single microfilaria of *Loa loa*. The examination was carried out in heparinized whole blood under a cover slip just as in the case of *C* and *D*, Fig. 2. The rate of travel was 0.37 mm. per minute, much faster than that observed for *Dirofilaria*. Notes made by the observer, D. L. A., are pertinent :

Laboratory of DR. F. W. O'CONNOR, Presbyterian Hospital, New York.

12th March, 1935. 11.25 a.m. Blood to the amount of 5 c.c. is drawn from a vein in the arm of the patient and heparinized. Tracings of courses of microfilariae would seem

to indicate much crossing and recrossing of path. This is not always true. Straight paths are often seen extending across the microscopic field after the preparation is several hours old. No unsheathed larvae are seen.

1.5 p.m. All larvae are as active as when the blood was first drawn. No unsheathed organisms are found in blood diluted with saline, in whole blood or in stained specimens. The tube specimen was transported to Boston without refrigeration.

Laboratory of Comparative Pathology, Boston, Mass.

13th March, 1935. 10.15 a.m. The microfilariae are as active as on 12th March. No unsheathed larvae are found.

14th March, 1935. The blood is contaminated and all microfilariae in the tube specimen are dead. Larvae on a slide under a vaseline-rimmed cover glass prepared yesterday are active. One larva has penetrated the vaseline with the anterior third of its body directed into the vaseline. The posterior two-thirds is lashing vigorously about and creating a fan-shaped area clear of red cells.

These observations show beyond possible question that sheathed microfilariae are capable not only of movement but of actual travel, and that their travel is forcible as indicated by the organism which penetrated the vaseline. So far as migration is concerned the microfilaria of *Loa loa* is even more effective than that of *Dirofilaria*, and it is reasonable to believe that the ease with which the latter organism traverses lymph nodes would be equalled by *Loa*. If the behaviour of *Loa* is a fair index of what one may expect from *Wuchereria*, it may be expected that microfilariae in lymphatics will pass to the blood stream at least as rapidly as does the lymph current in which they find themselves, and that healthy organisms will experience no serious check in passing through lymph nodes.

A further point, which will be treated in a second paper but which is pertinent here, is that if one cannulates lymphatics in various regions in a dog infected with *Dirofilaria*, microfilariae are found plentifully in the lymph. This means that these organisms readily get out of unbroken blood capillaries, traverse a certain distance in the tissues, and enter lymphatics. Experiments are planned to determine this point. At this time the presence of microfilariae (*Dirofilaria*) in lymphatics is merely further evidence of forcible movement on the part of these larvae. At autopsy of this particular animal adult *Dirofilaria* were found only in the right ventricle.

SUMMARY.

1. Experiments have been accomplished in which microfilariae (*Dirofilaria immitis*) have been perfused through the normal popliteal lymph nodes of dogs. It has been shown that there is no phagocytic filtration of the organisms and that they pass through the nodes with comparatively slight hindrance, differing from bacteria and even from the dog's own red cells which are phagocytosed in

the nodes, leaving the perfusate practically free of them when collected from the efferent vessel.

2. It has been shown that microfilariae of *Loa loa* although sheathed are more motile, *i.e.*, they travel farther per minute, than do those of *Dirofilaria* which possess no sheath.

3. By analogy it is suggested that microfilariae of *Wuchereria bancrofti* deposited in the lymph stream will not be measurably impeded by lymph nodes in their journey to the blood stream, and that if mechanically checked in nodes normal organisms will not suffer by such residence.

THE PERIODICITY OF THE MICROFILARIA OF *WUCHERERIA BANCROFTI*.

PRELIMINARY REPORT OF SOME INJECTION EXPERIMENTS.

BY

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Col. CLAYTON LANE has published several critical discussions regarding the mechanism of the nocturnal periodicity of the embryos of *W. bancrofti* and advanced the theory that this phenomenon was best explained by the daily disappearance and destruction of all the microfilariae in the blood with the appearance of a fresh brood of embryos the next night (LANE, 1929, 1931, 1933, 1934). This postulated a daily cyclical parturition of all the gravid females. O'CONNOR then showed, with his histological studies of massive serial sections of filarial tissue, that this cyclical parturition did occur. (O'CONNOR, 1931, 1932). As has been pointed out (MANSON-BAHR, 1929) the crucial point in LANE's theory is the actual survival time of the microfilaria.

With the encouragement and helpful advice of Dr. O'CONNOR and Col. CLAYTON LANE we have made a series of experiments injecting animals and human subjects with blood containing living microfilariae. At first our results were inconclusive, as have been the published reports of such experiments by others. (LOW *et al.*, 1933, MURGATROYD, 1933). However, in the light of our recent findings we can now explain our first results.

We make this preliminary report because we feel that our findings suggest an approach to the problem of filariasis from the angle of acquired resistance to the disease.

Case A. C., 8th March, 1933.

This subject was an advanced case of pellagra with mental symptoms. No clinical signs of filariasis. Blood negative for microfilaria. He was transfused into the median vein with 250 c.c. of citrated venous blood at 10.20 p.m. The citrated blood contained approximately 50 mf. per 20 c.mm. A film of blood from a finger tip of the opposite hand taken while the transfusion was still in progress, showed several microfilariae. Films made 24 hours later showed no microfilariae. Films made 48 hours after transfusion showed 2 microfilariae.

Case E. D., 20th June, 1933.

This subject was an advanced case of breast cancer having frequent haemorrhages from the ulcerated lesion. No clinical signs of filariasis. Blood negative for microfilaria. 400 c.c. of venous blood containing 56 mf. per 20 c.mm. was injected into the median vein. Blood removed from the median vein of opposite arm at 5 and at 15 minutes after the transfusion showed a few microfilariae. Blood examinations 12 and 24 hours after transfusion showed no microfilariae.

Case W. J., 29th July, 1933.

This subject had cirrhosis of the liver. No clinical signs of filariasis. Blood negative for microfilaria. 300 c.c. of citrated blood, removed at 9.6 p.m. and containing 58 mf. per 20 c.mm. was injected into the median vein at 9.38 p.m. In the 90 minutes following the transfusion, 20 specimens of blood were removed. A total of 440 c.mm. of capillary blood and 140 c.mm. of venous blood was spread on glass slides and 6 c.c. of venous blood was taken for examination of the sediment. In all this blood, only one microfilaria was found. Examinations of 500 c.mm. of venous blood at 12 hours and at 24 hours were also negative.

It was thought that perhaps the subjects of the above transfusions, although showing no clinical signs of filariasis, might, nevertheless, have a high degree of immunity to the parasites, since they were natives of a filarial district. Therefore, it was proposed to retransfuse a patient in the day with his own night blood.

Case C. A., 12th September, 1933.

This subject was in good health. His blood was positive for microfilaria and he showed enlarged inguinal and subinguinal lymph-glands and chronic filariasis of both testes. He did not have elephantiasis.

450 c.c. of his venous blood was drawn at 1.20 a.m., citrated and kept at room temperature (84° F.) until the next midday. The microfilariae were then found still active and within their sheaths. The blood was reinjected into the median vein at 11.16 a.m. At 11 a.m., just before the reinjection, two 500 c.mm. samples of venous blood contained 98 and 147 mf. respectively. After the reinjection, 500 c.mm. samples were taken at 1, 6, 15, 30 and 60 minutes and showed 138, 128, 116, 140 and 124 mf. respectively. Injection of this enormous number of microfilariae, over 2 millions, had produced no clear cut rise in the number of microfilariae in the day blood.

From the foregoing experiments it appeared that in some way in the body the microfilariae were filtered out of the blood very quickly and that only an occasional one made the circuit of the circulation. The results did not seem definite enough to warrant any conclusions.

In the period between the above experiments and the following ones, we learned how to detect small numbers of microfilariae with greater accuracy. If we had known this technique at first our results would have been more definite. This method of blood examination is as follows :

Method of Blood Examination.

Venous blood is drawn and kept from clotting by the addition of sodium citrate solution (1 c.c. of 2 per cent. solution to 10 c.c. of blood).

Capillary blood always shows more microfilariae than venous blood, but taking it is painful to the patient and only small amounts can be obtained.

For accurate or comparative counts, measured amounts of this citrated venous blood are spread on glass slides. These slides are laked in a 2 per cent. solution of formalin (40 per cent. solution of formaldehyde) which removes the haemoglobin and fixes the film to the slide so that it will permit staining. The films are then dried and stained 10 minutes or more with undiluted Giemsa stain.

For quick examination of larger amounts of blood, the blood is drawn and citrated as above and then divided into 1 c.c. amounts. Each of these is added to 10 c.c. of 2 per cent. formalin solution in a 15 c.c. conical tip centrifuge tube. The microfilariae are then collected in the tip of the tube either by centrifuging the tube or allowing it to sediment by gravity for 10 to 12 hours. The formalin solution laves the blood, preserves it, and prevents clotting of the sediment, and allows the sediment to adhere to the glass slide when spread out in a film later. After the sediment is thrown down, the fluid is decanted and the small drop of sediment in the tip of the tube is carefully collected with a fine capillary pipette and transferred to a glass slide. The drop is then spread into a film using the edge of the end of another glass slide as in making a thin blood film. The film is dried and stained a few minutes with undiluted Giemsa stain. In the stained film, which should be made thin, the microfilariae are very readily picked out. They are found stretched out straight, instead of being coiled as in the ordinary thick blood film, and the sheath stains pink, and there is no coloured background.

We have found this technique very satisfactory for detecting positive blood cases by examining the sediment of 1 c.c. of venous day blood. It is also particularly useful in making school-children surveys, for one can collect the blood in the daytime.

In our first experiments we found that the microfilariae were filtered out of the blood somewhere in the body. We next did some animal experiments to determine in just which particular tissue this took place.

Rabbit No. 3., 20th February, 1935.

Previous experiments had shown that the injection of any considerable amount of human blood into the rabbit caused clotting of the rabbit's blood in the heart and large vessels before death. In order to introduce a large number of microfilariae into the rabbit, we laked 20 c.c. of human blood with 0.1 per cent. sodium citrate solution and separated the microfilariae and suspended them in 20 c.c. of saline. This suspension contained 56 mf. per 20 c.mm. making a total of 56,000 mf. injected. The microfilariae were injected into the ear vein of the rabbit. After 15 minutes the rabbit was killed by a blow on the neck and the body was opened. The heart continued to beat for over 5 minutes. 500 c.mm. samples of blood were removed from the portal vein, inferior and superior venae cavae, renal vein, iliac vein, ear vein, pulmonary artery and heart. All specimens showed one or more microfilariae.

This experiment showed that the microfilariae were probably held up in the peripheral capillaries all over the body. This could be settled in a human subject by transfusing into a peripheral artery and noting if the microfilariae passed through the capillary ends of that artery.

Case D. G., 1st March, 1935.

This subject had a positive blood and showed filarial inguinal and subinguinal lymph-glands and bilateral orchitis and hydroceles, but no elephantiasis.

250 c.c. of venous blood was removed at 10.30 p.m. and citrated with 30 c.c. of 2 per cent sodium citrate solution. The citrated blood contained 72 mf. per 20 c.mm. The blood was kept at room temperature (about 84° F.) until the next midday. It was then examined and the mf. seen to be free and active and inside their sheaths. The blood was injected into the right brachial artery at 12 noon. Forty minutes before the injection, 500 c.mm. of venous blood from the right arm showed 21 mf. and from the left arm 24 mf. Ten minutes after the injection 500 c.mm. from the right showed 104 mf. and from the left 31 mf. At

3.30 p.m., 2½ hours after the injection, 500 c.mm. from the right showed 28 mf. and from the left 7 mf. Blood was also taken from the finger tips but the 20 c.mm. drops showed from 0 to 6 mf. which were too few to show any clear-cut difference in the blood from the two hands.

This experiment would indicate that the microfilariae were held up in the peripheral capillaries and could pass through only with great difficulty. But from this experiment, one could not say that the microfilariae recovered were the microfilariae injected.

Case S. G., 8th March, 1935.

This subject showed enlarged inguinal and subinguinal lymph-glands and chronic filarial orchitis and funiculitis but no elephantiasis and no microfilariae in the blood.

250 c.c. of venous blood was drawn at 10.30 p.m. from a matched donor and citrated. The citrated blood contained 68 mf. per 20 c.mm. The blood was injected into the right brachial artery at 10.40 p.m. At 10.55 p.m., 5 c.c. of blood was removed from each median vein and citrated. 500 c.mm. from each was spread on glass slides and the rest prepared for examination of the sediment. 120 c.mm. of capillary blood was also taken from each hand and spread on slides. Other 5 c.c. specimens of blood were taken from the right median vein at 12-24-36 and 48 hours and prepared in the same way. Also 120 c.mm. of capillary blood was removed each time. In all 3,000 c.mm. of venous blood and 720 c.mm. of capillary blood on slides, and the sediment from 24 c.c. of blood, was examined and not one single microfilaria was recovered.

The patient experienced no immediate reaction from the injection but at 16 hours he complained of generalized aches and pains. His temperature was 100.8° F. and it rose to 102° F. at 20 hours, returning to normal by the next morning. He had no chill, no nausea, no vomiting, no eruption or urticaria, and no asthmatic symptoms. There was no local reaction in the right arm or hand or any swelling or tenderness of the epitrochlear or axillary lymph-glands. The leucocyte count the morning after the fever was 7,200 with 85 per cent. polymorphonuclears and 7 per cent. eosinophiles.

It appears that this patient had a high degree of resistance to the microfilariae and that the microfilariae were held up in the peripheral capillaries and perished there. These findings suggest some very interesting possibilities. Are the peripheral capillaries the site where the microfilariae perish? Can one artificially produce such an immunity as this case showed, in a case showing only an early positive blood? Does this resistance to microfilariae also hold against filariae injected by the mosquito?

Case E. D., 18th March, 1935.

This subject showed no clinical signs of filariasis and his blood was negative. He was a native of another island where filariasis is not so prevalent as in St. Croix.

175 c.c. of blood from a matched donor was drawn at 11 p.m., citrated and injected into the right brachial artery at 11.10 p.m. The citrated blood contained 80 mf. per 20 c.mm. Blood removed from the right and from the left median veins showed microfilariae in the sediment. The next midday, 12 hours after the injection, 5 c.c. of blood was removed from each median vein. 1,000 c.mm. from each was spread on glass slides and the rest laked and centrifuged. In all this day blood only one mf. was found. 24 hours after injection 10 c.c. of night blood was removed from the right median vein and divided into 1 c.c. amounts which were laked and centrifuged and the sediments prepared. These showed 29-15-14-21-19-25-15-21-24-35 mf. The blood was examined each night during the first week and always found positive. The day blood was examined again on the fifth day and 3 microfilariae found in 10 c.c. The blood that night showed 16-14-8-12-10 mf. in 1 c.c. sediments. On the 14th night after the injection the sediments from 10 c.c. showed only one single microfilaria.

This experiment shows that in a non-immune host the microfilariae may live up to 14 days. It also shows that the microfilariae pass through the capillaries with difficulty and that they observe typical nocturnal periodicity throughout their life cycle.

All this suggests an explanation for the mechanism of periodicity. Perhaps the microfilariae do not pass through the capillaries in the daytime because they do not struggle so violently in the day blood as in the night blood.

Preparations of living microfilariae in day blood and night blood were placed side by side under the microscope and compared. The microfilariae appear distinctly less active in the day blood. And if this explanation is correct, one must remember that the day blood microfilariae collected do not show typical daytime activity but are the exceptionally active ones. This question needs much further careful investigation from different angles.

Why the microfilariae should be more sluggish in the day blood than in the night blood is unexplained. Perhaps cyclical parturition has something to do with it. The excretions of the female might contain a hormone. But this should not have obtained in our non-immune negative host (case E. D.). However, since he was a native we cannot be sure that he was altogether non-immune, for he must have had numerous opportunities for infection in his lifetime.

Case W. C., 29th March, 1935.

This was a subject with elephantiasis of the right leg and the right arm. He also had filarial orchitis and funiculitis and the inguinal and subinguinal lymph glands were slightly enlarged. His last attack of filarial fever was one year before and was in the right arm. His blood was negative for microfilaria.

125 c.c. of positive venous blood from a matched donor was drawn at 12.30 a.m. citrated and injected into the left brachial artery at 12.40 a.m. The citrated blood contained 140 microfilariae per 20 c.mm. Blood removed from the left median vein at 1 a.m. showed 7-7-16-19-12 microfilariae in the 1 c.c. sediments. Day blood removed at 11.30 a.m., 2½ days after the injection showed 3 microfilariae in the sediments from 10 c.c. *All three of these microfilariae were outside their sheaths.* Ten c.c. of night blood removed that night showed no microfilariae. This patient experienced no noticeable reaction whatever, local or general.

This experiment showed that the microfilariae may pass through the capillaries in a case of elephantiasis but that the survival time is shorter than in a less immune patient. The finding of microfilariae outside their sheaths in the blood stream needs further study.

SUMMARY.

- (1) A series of experiments is reported in which blood containing microfilariae was injected into non-filarial human subjects.
- (2) A method of blood examination for microfilaria is described.
- (3) Microfilariae were found to have great difficulty in passing through the peripheral capillaries.

(4) Microfilariae are less active in day blood than in night blood. It is suggested that because of this, they are not able to work their way through the capillaries in the daytime, hence the mechanism of nocturnal periodicity.

(5) In one subject the injected microfilariae lived 14 days and observed typical nocturnal periodicity.

(6) In another subject who showed clinical signs of filariasis, the injected microfilariae (which were injected into the brachial artery) never passed through the peripheral capillaries. This suggests an acquired resistance to the parasites, and that in an immune patient the microfilariae are stopped in the capillaries and perish there.

(7) In a subject showing elephantiasis the microfilariae survived only $2\frac{1}{2}$ days.

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THE NATURE OF THE DONOVAN BODY OF GRANULOMA INGUINALE.

BY

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Ever since DONOVAN's description of the intracellular bodies met with in scrapings from the ulcers of granuloma inguinale, these bodies have been the subject of much speculation. DONOVAN (1905) described them as diffusely stained by methylene blue, haemotoxylin, and the various modifications of the Romanowsky stain; and since they appeared to consist of an outer lightly stained area and an inner deeply stained condensed oval mass, he was inclined to regard them as protozoa; but he was unable to decide their exact grouping. Their protozoal nature, however, has not been accepted, and ARAGÃO and VIANNA (1913) put forward the view that they are schizomycetic and suggested the name *Calymmatobacterium granulomatis*. WALKER (1917) regarded them as capsulated intracellular diplococci belonging to the *Bacillus mucosus capsulatus* group, and claimed to have cultivated them on Sabouraud's medium. According to BERGEY's nomenclature, the name *Klebsiella granulomatis* was applied. GOLDZIEHER and PECK (1926) isolated an organism, Gram-negative and non-capsulated, which produced granulomatous lesions in rabbits: for this they suggested the name *Bacillus venereogranulomatis*. Successful cultures of the Donovan body have been claimed by MCINTOSH (1928) and also by GAGE (1929). CASTELLANI and MENDELSON (1929) consider that, in these cultures of the Donovan body, the organism that is grown is a capsulated variant of

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the *Bacillus lactis aerogenes* group and not of the *B. mucosus capsulatus* group. When injected into laboratory animals, abscesses were produced. DE MONBREUN and GOODPASTURE (1931a) have not succeeded in cultivating any organism morphologically similar to the Donovan body in spite of extensive attempts on various laboratory media. In three cases, however, Ducrey's bacillus was grown. They have produced chronic lesions in monkeys by implantation of material containing the intracellular forms of the organism (DE MONBREUN and GOODPASTURE, 1931b), whereas they point out that, so far, attempts at transferring the disease by inoculation of cultures have failed.

In our own attempts at cultivation (MENON and KRISHNASWAMI, 1933), we have recorded successful cultures in ten out of twelve cases. The organisms were isolated in pure cultures in eight cases. Of the eight strains studied, all fermented glucose and maltose with the production of acid, while gas was produced in two strains. Saccharose and salicin were also fermented by the majority of strains, but no gas was formed. Faint acidity was noticed in litmus milk. Lactose was not fermented in any of our series, though BERGER (1926) has pointed out that the production of acid and gas in dextrose, lactose and saccharose is the distinctive feature of *Klebsiella granulomatis* and *Klebsiella pneumoniae*. On the other hand, SMALL and JULIANELLE (1923) are not inclined to lay much stress on the fermentation reactions of this group for purposes of identification. Two strains studied with other carbohydrates showed that fructose, laevulose, galactose, raffinose, dextrin, arabinose and xylose were all fermented. Inulin, inosite and adonite were unaffected, though it has been pointed out that inosite fermentation is the characteristic feature of the *B. lactis aerogenes* group.

The colonies on agar are small, round and convex, about 1 mm. in diameter, in 24 hours, having a translucent, shiny appearance and showing a tendency to confluence. The growth in subcultures was profuse and of a yellowish brown colour. The colonies were viscid and easily emulsifiable. The edges were slightly wavy and a central bossing was noticeable in some strains. The central translucency was lost in 48 hours and the colonies became dry. A distinction could be made between shiny translucent colonies and a drier roughish more opaque type. They more or less corresponded to the smooth and rough variations. A uniform turbidity was noticed in broth in 24 hours and a slight deposit occurred in 3 days. Pellicle formation was not met with. The Voges-Preskauer reaction was negative and no indol was formed. In the frequent absence of capsular material in cultures and sometimes in smears, the organisms seem to differ from the mucoid encapsulated group, whereas the appearance of the colonies and biochemical reactions suggest some relationship. In view of the uncertainty of grouping between the *B. lactis aerogenes* and mucoid encapsulated groups, we have suggested the name *Bacillus donovani*.

The questions that naturally arise are, whether different organisms have been cultivated by different observers, whether the organisms identical with the

Donovan body have been cultivated and whether the Donovan body is really responsible for the lesion.

In this connection, a study of the morphology of the Donovan body may be of interest. These are small ovoid bodies, about 1 to 1.5μ in length, 0.5 to 0.7μ in thickness. In general, the shape is that of a small bacillus, the ends being slightly rounded and ovoid, while the maximum thickness is seen in the middle. In smears, each body is surrounded by a more or less clear ovoid area, which was originally regarded by DONOVAN as the cytoplasm of the parasite. Typically, the organisms are found inside cells, mostly the large mononuclear cells and also epithelial cells of the deeper layers, but in the latter, they are not quite so distinctly demarcated as in mononuclear cells. Occasionally, polymorphonuclear cells appear to be parasitised. Extra-cellular clusters are also met with, where the containing cell has disintegrated or had undergone rupture. The organisms are diffusely stained by most of the protozoal stains such as Leishman or Giemsa. We have found that they are best stained by any of the modifications of the Gram stain with weak carbol fuchsin as a counter stain. In such preparations, the bacillary shape is very definite and each rod-shaped or ovoid mass appears to be surrounded by an oval or irregular area which looks like mucinoid material formed by the organism. Inside cells, this capsular material fuses together, so that the protoplasm of the affected cell appears foamy around the organism. A study of the affected cell clearly demonstrates that the organisms live inside the cells (as shown in the Plate) and undergo multiplication there. Phagocytic disintegration of the organism is not a feature. Multiplication occurs inside the cell by the fusion of the capsular mass of neighbouring organisms to form cyst-like spaces in which the dividing forms can be met with in clusters. The early division forms vary in shape from definite bacillary forms which show bipolar staining, probably from central constriction of the body, to diplococcoid forms where division is complete, but the organisms remain fused together by adhesion of capsular material. Following intracystic multiplication, the cyst may rupture and then forms might be thrown out into the surrounding tissue. Diplococcoid forms are commonly met with after division. While cyst formation and intracystic development are frequently met with in many cases, the affected cell may appear loaded with organisms and division may occur in scattered foci, the protoplasm of the cell gradually undergoing disintegration. This seems to be the result of heavy parasitization of the affected cell, whilst cyst formation appears to be the result of a slight infection. With disintegration of the cell or rupture of the cyst, the extra-cellular clusters appear as capsulated bacilli or diplococci. The capsular material is not condensed to form a true outer cover for the organism, since capsule staining gives variable results. While capsular material is usually found in the intracellular forms during division inside cysts, cells are met with which show organisms with very little capsular material (as shown in the Plate). It appears that, in extracellular forms, the capsular material is gradually lost. In smears,

one can easily demonstrate clumps of organisms sticking together in a mucin-like material in the neighbourhood of disintegrating nuclei of mononuclear cells. A gradual diminution and disappearance of this capsular material can be seen in smears around scattered organisms. Bacillary forms with commencing division may show a central unstained area with staining at either pole and a resemblance to Ducrey's bacillus is thus brought about, but the typical chain forms of the latter organisms are not met with.

With regard to the lesions produced by the organism it was pointed out, by one of us (MENON, 1933) that the external genitalia form the site of predilection and infection spreads from here to the genito-crural folds. A study of the organisms in the tissues could only be carried out if they were stained by Weigert's modification of the Gram stain, since haemotoxylin stained sections do not show the intracellular forms distinctly. Superficial epithelium of the muco-cutaneous junctions is first invaded and the infection of the mononuclear cells is a later event when the granuloma begins to extend. A remarkable feature is the comparative rarity of implications of the deeper tissues in early stages of the disease. In our experience, smears taken from the deeper tissue do not often show the organism. The parasites would thus be regarded as capsulated organisms which have a special affinity for moist surface epithelium and subsequently invade the mononuclear cells.

A study of the attempts at cultivation of the organism by various observers shows the very large percentage of positive results that have been claimed. A critical study of the capsulated diplococcus described by WALKER, the bacilli resembling Ducrey's bacillus reported by others, the non-capsulated *Bacillus venereogranulomatis* and the small bacillus described by us would seem to indicate that all these organisms are only different stages in the process of division and development of the Donovan body; their ready growth on Sabouraud's maltose agar, the formation of small mucoid-looking round colonies which gradually show a slightly wavy margin, all these suggest a similarity.

In Norris medium, we have noticed that some capsular material is retained in primary cultures; but, on subculture, this is almost completely lost so that no capsule can be demonstrated by Hiss's method or Malone's method. In

DESCRIPTION OF PLATE.

THE DONOVAN BODIES OF GRANULOMA IN SMEARS STAINED WITH GRAM STAIN.

(Zeiss 1/12 objective, $\times 10$ ocular.)

1. The early stage of parasitisation of a mononuclear cell.
2. Cyst formation by the secretion of capsular material.
3. Heavy infection without cyst formation.
4. Cyst formation in one solitary focus. Note the diplococcoid and bacillary forms.
5. Rupture of a cyst with liberation of diplococcoid forms.
6. Disintegration of the cell-protoplasm with liberation of bacillary and diplococcoid and bipolar stained forms, all capsulated.



THE DONOVAN BODIES OF GRANULOMA.

subcultures on maltose agar, all traces of capsular material are lost and this factor may probably account for the difference in morphology noticed by different workers.

With regard to attempts at experimental transmission of granuloma by inoculation of cultures, it is striking that most workers have reported ulcerative lesions and abscesses in various laboratory animals, but the actual reproduction of the lesion has not been clearly demonstrated. On the other hand, DE MONBREUN and GOODPASTURE (1931a) have established chronic lesions in monkeys by inoculation of tissue containing the intracellular forms of the Donovan body. Our own experiments on guineapigs by intradermal inoculation of cultures showed only slight superficial reactions which healed. On subcutaneous inoculation in the skin of the abdomen, however, small ulcers developed on the sixth day, but these gradually scabbed over and healing was complete by the 20th day. From these ulcers, smears showed typical intracellular and extracellular clumps of the organisms and they could be recovered in culture. White rats are much more susceptible and severe necrotic and ulcerative lesions were produced on subcutaneous inoculation. With young white rats, severe local necrotic reaction was followed by general septicaemia and death in about twenty-four hours. The organisms were recovered from the heart, blood and spleen. We have succeeded in producing similar ulcers in a dog but a chronic lesion similar to human granuloma has not yet been established. Records of the experiments are summarised in the appendix.

It has been objected that the lesions produced by inoculation of cultures are abscesses and not granulomatous ulcers. In our studies (MENON and NATESAN), we have noticed that the primary lesion in human granuloma starts as a pustule on the genitalia. This breaks down and becomes an ulcer where the Donovan organisms can be demonstrated. This has been called the primary lesion or the first stage of the disease. If one were to assess the result of this experimental work, one is driven to the conclusion that the organisms met with in culture differ in their pathogenicity from the intracellular forms of the Donovan body. We are tempted to assume, that the chronicity of the lesion may be due to the presence of the capsular material in the intracellular forms. As this capsular material is lost in artificial cultures, the organisms seem to produce much more acute reactions. It may be argued that the Donovan body itself has not been cultivated, but the traces of capsular material that could be defined in primary cultures, and the successful cultivation of one organism in a large percentage of cases are against this view.

With regard to the association between the Donovan body and granuloma inguinale, the positive findings in a large series of cases by numerous workers, indicate a direct association between the lesions and the organism. In our own examination of smears from ulcers met with in various types of lesions, in different parts of the body, we have never found a similar organism except in the typical ulcers of this granuloma.

SUMMARY AND CONCLUSION.

The Donovan bodies met with in granuloma inguinale are intracellular capsulated organisms morphologically similar to capsulated bacteria. The organism appears to be a small Gram-negative bacillus, which, during the stages of division, shows diplococoid forms and forms showing bipolar staining. The organism lives inside the epithelial cells and large mononuclear cells. In the latter, the stage of growth and development inside cystic foamy areas in the protoplasm may be made out. With the rupture of the cells, the capsulated organisms are thrown out into the surrounding tissue. Organisms morphologically similar to the Donovan body can be cultivated on Norris medium and maltose agar, but with subcultures the capsular material is lost. These organisms produce more acute ulcerative lesions than are met with in granuloma inguinale, but the typical intracellular forms of the Donovan body can be demonstrated in the lesions where the healing is delayed. The very close and constant association between the Donovan body and granuloma inguinale and its absence in other types of ulcerative lesions afford strong presumptive evidence of an aetiological relationship. Some positive evidence is brought forward to show that the organisms obtained in culture are identical with the Donovan body.

APPENDIX.

EXPERIMENTS ON GUINEAPIGS.

Experiment 1.—Patient, Mrs. G. A., with granuloma of the groin and perineum. Smear positive for Donovan organisms.

1932, June 14th. 24 hours' culture from agar inoculated intradermally into the groin of Guinea-pig 7.

1932, June 15th. Small tender lump formed in the groin.

1932, June 20th. Small ulcer of the size of a pea, at the site of injection.

1932, June 22nd. Ulcer shows signs of healing.

1932, June 30th. Small painful nodule had formed round the ulcer; lymph glands not enlarged; contents aspirated and cultured on broth showed Gram-negative diplobacillary forms. Subcultured on agar; pin head sized colonies with a translucent slightly wavy border. Smears showed Gram-negative small bacilli, but no capsule staining can be made out with Malone's method; otherwise organisms indistinguishable from Donovan bodies. Fermented glucose, saccharose and maltose with the production of acid.

Experiment 2.—Patient, same Mrs. G. A.

1932, July 1st. Broth culture injected both intracutaneously and subcutaneously into Guinea-pig 8, in right and left groin.

1932, July 4th. A small abscess had formed at the site of subcutaneous injection; this had become converted into a small ulcer with a smooth base; no reaction at the site of intradermal injection.

1932, July 16th. The ulcer shows a granulating base; the organism recovered from the ulcer by culture on maltose agar.

1932, July 22nd. The ulcer shows sign of healing.

Experiment 3.

1932, July 9th. Subculture from Guinea-pig 7, inoculated into male guinea-pig, full grown No. 9.

1932, July 11th. Large nodular swelling at the site of subcutaneous inoculation and small nodule at the site of intracutaneous injection.

1932, July 18th. Large areas of induration, but no abscess at the site of subcutaneous injection and a small nodule of the size of a bean at the opposite side.

Experiment 4.—Patient, Mrs. B. G. A., with typical granuloma involving the vulva, with a positive smear.

1932, July 22nd. Inoculation of agar culture into Guinea-pig 10. Smooth type of colony into left groin and roughish type of colony into right groin.

1932, July 26th. An ulcer had formed on the right side, but had scabbed over, while on the left side the ulcer was well marked and indurated with a large nodule underneath. No lymph glands palpable.

1932, August 12th. Healing has commenced.

Experiment 5.—Patient, Mrs. R. A., with well developed granuloma of the groin and vulva. Smear positive for Donovan organisms.

1932, November 5th. Injected emulsion of young agar culture intraperitoneally into Guinea-pig 11. Died in twelve hours.

1932, November 6th. Postmortem examination of guinea-pig shows peritonitis and septicaemia. Organism recovered from peritoneal cavity shows involution forms, slightly longer and bacillary forms without any trace of capsular material.

Experiment 6.—Patient, Mr. G. C., with large granuloma of the groin with healed penile lesion; smear positive.

1933, March 28th.—Young blood agar culture inoculated subcutaneously into the groin of Guinea-pig 12.

1933, March 29th. Diffuse brown induration at the site of inoculation. This persisted for two weeks and developed into an abscess.

1933, April 27th. Puncture fluid from abscess shows Gram-negative bacilli similar to the Donovan organisms. Cultures on agar show identical colonies. The organisms fermented glucose, saccharose and maltose with the formation of acid in litmus milk.

EXPERIMENTS ON WHITE RATS.

Experiment 1.—Patient, Mrs. R. A., with granuloma of groin and vulva; smear positive.

1932, November 5th. Young agar culture inoculated under skin of white rat, full grown female No. 5.

1932, November 8th. Small area of skin is indurated and exudes a little serum at the site of injection. The next day, this has developed into a well marked ulcer with an indurated base and a slight discharge. The ulcer measured $\frac{3}{4}$ -in. by $\frac{1}{2}$ -in.

1932, November 11th. Cultures from the ulcers show typical colonies, slightly roughish; microscopically, typical Gram-negative bacilli. In broth, there is a diffuse turbidity.

Smear from the ulcer shows typical forms of the Donovan body, both intracellular and extracellular clusters.

1932, November 25th. Ulcer shows signs of healing.

Experiment 2.—Patient, Mr. G. C., with granuloma of groin and healed penile lesion, smear positive.

1933, March 23rd. Inoculation of emulsion from blood agar slope into subcutaneous tissue of abdominal wall of young half-grown white rat No. 6.

1933, March 24th. White rat died in 24 hours; postmortem shows general septicaemia, congestion of liver, spleen and kidney; organisms recovered from the heart, blood and spleen. Extensive necrosis and oedema at the site of injection.

Experiment 3.

1933, March 25th. Injection of same culture as in Experiment 2 into another young half-grown white rat No. 7. White rat died in 24 hours; postmortem appearances, similar.

EXPERIMENT ON A DOG.

Patient, Mr. L. N., with extensive granuloma, involving the penis, scrotum and groin ; 7 years' duration ; smear positive for Donovan organisms ; Fric's test negative.

1934, May 28th. Emulsion from 24 hours agar culture, inoculated into left thigh subcutaneously.

1934, May 30th. A soft swelling—an abscess at the site of injection of the size of a hen's egg.

1934, June 2nd. The abscess had burst and left an ulcer about $\frac{1}{2}$ -in. in diameter, oval in shape, a sharp margin and a smooth base.

Smears from the ulcer show *intracellular* and *extracellular* forms of Gram-negative bacilli and diplococcoid forms.

1934, June 14th. Ulcer shows no signs of healing. Organisms recovered on culture ferment glucose, saccharose and maltose with slight acidity in litmus milk.

1934, June 26th. Ulcer shows signs of healing.

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AINHUM DISEASE AND THE ANAESTHETIC TYPE OF LEPROSY.

BY

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Ainhum is a disease on the etiology of which several theories have been advanced. According to the description in *Manson's Tropical Diseases* the word itself is derived from the Naga dialect and means "to saw" or "to cut." In this affection, which is usually bilateral, a constricting ring, with the appearance of a tight ligature, occurs at the root of the little toe. The disease is very chronic and runs a course of from 5 to 10 years, the toe eventually falling off or being surgically amputated. The condition is painless and causes no inconvenience to the patient except in the later stages when the toe may be hanging loose and be liable to interfere with walking. So far as is known the white-skinned races are immune, and the condition occurs chiefly in Negroes and Indians. I have only seen one case in Malaya, in an Indian Tamil who is being kept under observation. There appears to be a tendency for the condition to be familial. According to DUPREY it is frequently accompanied by severe loin pains in the earlier stages. In our case, the patient attended hospital on account of pains all

over the body and the ainhum was observed by Dr. KELLY during the course of routine examination, and my attention was drawn to it. The presence of these body pains suggests a possible nerve lesion and that the condition may be trophic.

The object of this communication is to draw attention to the similarity of the lesion which may occur in the anaesthetic type of leprosy. Within the past 3 months I have seen an almost exactly similar condition affecting the great toe in two leprous patients ; but leprosy may affect all or any one of the digits, while ainhum rarely affects any other than the little toe.

According to MANSON-BAHR, section of the ainhum lesion shows that the superficial fascia around the constriction is hypertrophied and fibrous and the bone is infiltrated with fatty matter. The pathological amputation may occur either through a joint or through the bone. The presence of this fatty degeneration is strong evidence of a trophic lesion, but it is difficult to say with certainty whether the atrophy is due to secondary pressure on the vascular supply or primarily through nerve involvement. One might expect, however, that a secondary vascular constriction would produce atrophy at the extremity of the digit. In actual fact the toe beyond the constricting ring maintains its vitality until the latest stages, thus suggesting a nerve lesion similar to that which occurs in leprosy.

Another theory concerning the etiology of ainhum is scleroderma. The wearing of toe rings has also been blamed, but in Tamils, who frequently wear metal or string rings on the great toe, I have not observed a single case of pathological constriction. MANSON suggests that it is due to wounds of the root of the little toe from walking barefoot through grass or jungle : such wounds set up a chronic fibrosing irritation which encircles the toe in a sclerosing ring. This theory, however, does not fit in with the familial tendency, nor with the selective nature of the disease as the other toes are so rarely involved. Wounds in this situation usually become more acutely septic than the mild ulcerative lesion which occasionally accompanies the ainhum. It is also peculiar that ainhum affects adult males ; but rarely women or children, although one might reasonably expect that children running barefoot would be the chief sufferers if the condition were due to frequently repeated minor wounds of the toe.

In our case the patient was a male Tamil, age 28 years, who had resided in Singapore for only 2 years : prior to emigrating to Malaya, he had lived in southern India since birth. His complaint was one of vague shifting pains in his body of 2 months' duration. There was nothing of note in any of the major systems, and tests for nerve lesions failed to elicit any anaesthetic areas. Ear clips were examined for *Bacillus leprae* but the findings were negative. Figure 1 shows the constricting ring on the little toes. The Wassermann reaction was + + but a positive Wassermann is the rule rather than the exception in this class of native. The pains cleared up under rest and analgesics, and he was discharged but returned 5 months later with similar symptoms. These pains



FIG. 1.

FIG. 2.



FIG. 3.

Photograph of the feet of an adult male Tamil to show the bilateral constricting ring at the root of the little toe.

Photograph of the constricting ring round the great toe in a case of anaesthetic prosy. Note the marked resemblance of the ring to that in Fig. 1.

Constricting ring round the great toe in an adult male Hylam Chinese suffering from leprosy.

may have had no connection with the ainhum since vague pains occur in most people's lives during some period or other.

Figure 2 shows an annular constriction of the great toe occurring in a case of leprosy and one cannot fail to be impressed with the macroscopic similarity of the lesion. This patient was a Teochew Chinese female, age 16 years. She complained of a sore on the sole of the left foot which had been present for 2 months. On examination, two perforating ulcers were found on the sole of the foot. The left leg was swollen and a scaly dermatitis extended as far as the knee. There was loss of light touch and temperature sensation over the lateral side of the leg and dorsum of the foot, and loss of the sensation of pain over a small circumscribed area on the lateral side of the left knee and left foot.

A periarterial sympathectomy was attempted in Hunter's canal but the wall of the artery was found to be soft and very badly diseased. The peroneal nerve was exposed on the neck of the fibula and scrapings taken. *B. leprae* were found in profusion. Ear clips were negative. She was discharged in 28 days with the perforating ulcers very much improved but no change in the constriction of the great toe.

Figure 3 shows a similar condition in a male Hylam Chinese, age 34, who had resided for 19 years in Singapore. On admission he complained of headache and constipation for 15 days, and incidently mentioned a sore on his right foot of 3 months duration. The sore had apparently healed but had broken down 3 weeks prior to admission. On examination he was found to have a discharging sinus over the dorsum of the fifth metatarsal. The little toe had dropped off some years earlier. There was a marked constriction round the base of the great toe. On further questioning he admitted a perforating ulcer on the outer side of the sole of the foot of 15 years duration, but as it was not painful he did not trouble about it. The right foot and leg up to the knee were anaesthetic to light touch. The right peroneal nerve was thickened and nodular. Ear clips were made and found positive to *B. leprae*. An X-ray showed marked destruction of the right fifth metatarsal bone.

A periarterial sympathectomy was performed in Hunter's canal and the condition improved for a few days. Then a superficial abscess formed on the dorsum over the fifth metatarsal 9 days after operation. The abscess was incised and some bone sequestra evacuated. He was discharged in 3 weeks at his own request with the ulcer condition very much improved but no change in the ring constriction of the great toe.

CONCLUSION.

In my opinion there is a marked similarity in the constricting pathological amputation of ainhum and that of leprosy. I believe for the reasons given above, that ainhum is a trophic lesion due to a very chronic irritation of the nerve, the nature of which has not yet been elucidated.

A "FOLLOW UP" AFTER EIGHT HUNDRED SPLENECTOMIES FOR EGYPTIAN SPLENOMEGALY.

BY
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On bringing my splenectomies, referred to in a previous note*, up to a total of 800, I decided to celebrate the occasion by going out to the villages to find a few of the men I had photographed before operation.

	Patient's Name.	Urine.	Faeces.
1	El Sayed Shabara	Negative	<i>Ankylostoma</i> & <i>Ascaris</i>
2	Abdou Sirag Toaiama	<i>B. haematobium</i>	<i>B. mansoni</i> & <i>Ascaris</i>
3	Messad Abou Hilali	Negative	<i>B. mansoni</i>
4	Abdou Ali el Ghazi	Negative	<i>Ascaris</i>
5	Moh. Abou el Maati	Pus cells	<i>B. mansoni</i>
6	Hammed Raggab	Red blood corpuscles	<i>B. mansoni</i>
7	Moh. Soliman el Hifnawi	Negative	<i>Ascaris</i>
8	El Shirbini Ali Sherif	<i>B. haematobium</i>	Negative
9	Ahmed Moh. Abd el Fattah	<i>B. haematobium</i>	<i>B. mansoni</i>
10	Salmi Salama	Negative	<i>Ankylostoma</i>
11	Mostapha Ali Yousef	Negative	<i>B. mansoni</i> & <i>Ascaris</i>

Urine and faeces examinations were carried out and the results in eleven cases are tabulated above.

*STIVEN, H. E. S. (1931). Geographical distribution of cases of Egyptian splenomegaly
Trans. Roy. Soc. Trop. Med. & Hyg., xxv, (1), 77.

On seeing these men and asking after their health, work and general condition, I was much impressed by their increase in stature and musculature, and it appears that the removal of the enlarged spleen definitely improves their health and physique, in spite of the re-infection by parasites. I say re-infection, because my rule is always, before splenectomy, to give a complete course of tartar emetic for the *Bilharzia*, and carbon tetrachloride for the *Ankylostoma* and *Ascaris* infections.

I have to thank the district medical officers and officials who helped me to find my old patients.

A COMMENTARY ON THE DIARY KEPT BY
PATRICK MANSON IN CHINA AND NOW CONSERVED
AT MANSON HOUSE.

BY

PHILIP MANSON-BAHR.

No account of the life work and discoveries of Sir PATRICK MANSON would be complete without some reference to the extraordinary book which constituted his scientific diary during those fruitful years spent in Amoy and Hongkong. It is probably safe to prophesy that this collection of observations will be regarded as of great value by future students of medical history.

MANSON commenced his Diary in June, 1877, and continued to make entries therein with commendable regularity for another twenty years. The Diary is a plain ledger, bound in buckram, and appears to have been acquired by MANSON on his advent to Amoy, for on the outer cover can still be deciphered "Imperial Maritime Customs, Amoy." The book measures 13 by 8 inches and contains 612 closely-written pages.

In these pages there are gathered together scattered observations on the main subjects for which MANSON's name became world-famous. It was apparently his habit to record his observations and experiments daily in an orderly fashion and to keep transcripts of all the letters he wrote to correspondents such as TIMOTHY LEWIS, SPENCER COBBOLD, LEUCKART, LISTER and the *Lancet*. Inserted too, between these pages, are numerous original drawings. The Diary is therefore replete with scientific, historic and human associations. Naturally the main interest radiates round his first-love—filariasis.

The first fifty pages are largely devoted to observations on the clinical manifestations of filariasis. There are accurate accounts of lymph-scrotum, in some cases of which he discovered embryo filariae both in the blood as well as in the lymph, whilst in others he was able to trace the beginnings of elephantiasis. On page 10 is recorded an interesting experiment (19th August, 1877), in which an attempt was made to infect a monkey with filaria by feeding it on a banana in which were inserted twelve mosquitoes which had previously been fed on filariated blood. At this period too, there are to be found numerous observations on the association of filaria with hydrocele and also the frequency of the coincidence of filariasis and leprosy.

We know, from the reports which MANSON published from 1871 to 1878, the interest which the study of leprosy had aroused in his mind, so that it is not surprising to find on p. 76 a record of the discovery of what appears to be the bacillus of leprosy (6th February, 1879). He says that in lymph expressed from the tubercle of one, Tui Hse, he discovered micrococci. These are figured together with red blood corpuscles for comparison, and it is more than possible that the "micrococci" were true leprosy bacilli. Then follows an account of his attempts to grow the organism by inoculation of infective material into hens' eggs which were subsequently incubated under a hen. The eggs were examined daily and a careful record of findings kept; but he apparently succeeded only in isolating "zoogloea masses" of fungi from the eggs. At the same time he appears to have discovered, quite independently, the method of growing micrococci in capillary tubes filled with beef juice and milk. Moreover, at this time he attempted to reproduce leprotic lesions by inoculating "leprosy juice" under aseptic precautions directly into the aqueous humour of fowls (pp. 100-104). From time to time there are other references to his concern regarding the true etiology of leprosy. On p. 446 (1884) we find it recorded that he was able to stain and recognize the bacillus of leprosy.

Amongst the many original discoveries made by MANSON at this most fruitful period of his career, there was hardly one more romantic than that of the lung fluke—*Paragonimus*—originally known as Ringer's fluke—*Distoma ringeri*. We find, on pp. 190-191, under the heading of "Haemoptysis—ova of entozoan in sputum" (24th April, 1878): "... came to consult me about an eczematous eruption he has on both cheeks and chin and also on both legs. ... I observed while he was speaking to me that he hawked up a small quantity of reddish sputum and that his voice was rather rough and loud. I examined the sputa which in part were made up of pellets of rusty pneumonic-like sputa. ... Under the microscope were ... many oval ova, dark red, blood-stained, measuring $1/330 \times 1/500$ inch, granular on the surface, having a lid on flattened open end at one long diameter. Contents are very indistinctly marked, but certainly no distinctly differentiated embryo is visible." On p. 196 there is the actual record of the discovery of the parasite—the lung fluke—*Paragonimus westermanni*.

The patient was a Portuguese suffering from a thoracic aneurism who, after leaving Amoy Hospital, returned to Formosa where he died suddenly from rupture of the aneurism into the pericardium. Dr. RINGER, who made the postmortem, wrote "The parasite I found, after making a section, lying on the lung tissue; whilst alive, a number of young (ova) escaped from an opening in the body." The specimen was preserved in spirit and forwarded to MANSON, who found, in the sediment of the bottle, ova similar to those he had already seen in the sputum of the Chinese patient noted above. He next examined (p. 197) the sputum from 20 Chinese patients in Amoy with a negative result. This subject is continued further on p. 317 to which is affixed a manuscript letter from Dr. E. BAEZ of Tokio (dated 19th February, 1880) recording the

despatch of sputum from Japan containing eggs which MANSON found to be identical with those from his Formosan case.

Next (pp. 320-342) are recorded minute experiments which he made in an attempt to hatch out these ova; this MANSON found to be by no means so easy as he had anticipated, and it was his failure to observe any noticeable changes in the contents of the egg, which led him to speculate still further on the possible life-history of this parasite. As will be seen in the sequel, he came extraordinarily near the mark. He commenced his observations on 14th, February 1879, by placing the sputum in various glasses; he washed it daily with fresh water for many days, but no change was observed in the egg contents till 9th August, when one egg was found with a developing embryo inside it. On pressure on the shell, the ciliated embryo escaped. Two days later other eggs hatched, the embryos rushing off from the eggs as globular ciliated rotating balls. The anterior extremity of the embryo was provided with a papilla or beak-like rostellum.

Later, he found that development was favoured and the escape of the miracidia accelerated by the daily addition of fresh water and by keeping the temperature constantly at 60° F. The most surprising fact, however, about these experiences was his forecast of the probable life cycle of the lung fluke. Thus, we find, on p. 342 in a letter from a correspondent in Hongkong (R. R. HUNGERFORD, 21st October, 1881) the information that various kinds of fresh water snail were suggested as intermediary hosts for the lung fluke. It appears that Mr. HUNGERFORD had made a special study of fresh water snails and from a collection of shells which he despatched to MANSON, we find that *Melania libertina* (GOULD) was selected as being the most likely one to be concerned with the transmission. HUNGERFORD, in his letter, remarks upon the wide distribution of this snail and concludes as follows:—"On the whole, I think *M. libertina* must be your friend; he is a hardy beast. It will be something in favour of my hobby if it helps to clear up a doubtful point in the natural history of flukes." But it appears that MANSON's idea at this time was that the infection was contracted actually by eating these molluscs, for on pp. 420-421 and pp. 431-433 are recorded examples of this disease in man (1st December, 1883) in which the infection is supposed to have been contracted in this way. One patient is stated to have *eaten* fresh water molluscs and to have commenced to cough shortly after partaking of the first dish of fresh water snails. He formerly said that this mollusc was much eaten in Formosa, a practice which had been discontinued when a certain man had said that the snails fed upon dead men's flesh. This information, none of which appears to have been previously published, constitutes a striking forecast of the wonderful life-history of this parasite, subsequently elucidated by NAKAGAWA in 1916 (35 years later).

Students of the life of MANSON will naturally be most interested in his discovery of the phenomenon of filarial periodicity and they will find here much food for reflection. From pp. 208-223 there are columns of statistics kept in

July and August, 1880, recording the temperature of the air and the barometric readings, together with the body temperature, pulse rate, hours of sleep and nature of the food of a number of Chinese patients. The number of microfilariæ in the blood at different hours of the day is also noted. This section demonstrates the careful manner in which MANSON's observations were then recorded. These enabled him to draw up with his own hands the graphic chart of the phenomenon of periodicity, the original of which is now preserved in the museum of the London School of Hygiene and Tropical Medicine.

Towards the end of 1878 and at the beginning of 1879 MANSON commenced correspondence with the leading helminthologists of the day regarding the pathology of filariasis and on pp. 123-130 there is a copy of a long letter to Dr. SPENCER COBBOLD, F.R.S.; in this letter, dated 20th June, 1879, are to be found the first recorded examples of filarial periodicity. He had found that one of his gardeners, who came from a filarious district, was heavily infected and that the microfilariæ appeared in his blood with remarkable punctuality at night time. It was this man whom he used for further experiments; the letter ends as follows:—"It seems to me that LEWIS, by his great discovery, has opened a new field in tropical pathology. The interest and importance of *F. Bancrofti* and *F.S.H. [Filaria sanguinis hominis]* is by no means exhausted. . . . Men like myself in general practice are but poor and very slow investigators crippled as we are with the necessity of making our daily bread."

On p. 132 we find a record of the examination of the various organs of dogs for numbers of microfilariæ in cases of *Filaria immitis* infection. A dog had been killed with prussic acid and by far the largest number of embryos were found to be present in the lungs. MANSON, therefore, came to the conclusion, at this early stage of his investigations, that the embryos became fixed in the pulmonary tissues during the daytime, for he had previously ascertained that in this infection of the dog a rough kind of periodicity is maintained; that is to say that the embryos are more numerous in the blood during the hours of night than they are in the daytime.

This interesting question of periodicity is pursued further on pp. 234-250 in another letter addressed to SPENCER COBBOLD and dated 25th August, 1880. This communication is still worthy of the close attention of students of this remarkable phenomenon. MANSON considered that it well deserved the attention of physiologists. For, he writes, "could we ascertain what the subtle influence is that sets the creatures circulating in the blood stream and arrests them with such military punctuality, we probably would let new light in on many an obscure problem, both in physiology and pathology. It was with the intention of providing myself with a standard with which to compare the result of observation and experiment that I prepared the chart I sent you. . . . It is evident that the power which fixes them and lets them loose operates independently of the sleeping state. It is associated with the advent of night, but not of sleep. . . . Whatever the cause may be, it certainly operates through the body, the medium

in which the parasites are, but I very much incline to think, that though operating through the body, it is placed outside of it.

"Of one thing we may be quite certain, that from the fact of the periodicity being one of 24 hours, its remote cause is the rising and setting of the sun, or rather, the altered relation of the earth's surface recurring every 24 hours. Of another thing we may also be certain, that the immediate cause is applied between the hours of 5 and 7 p.m. What then, is the phenomenon in Nature which, depending on the position of the earth's surface to the sun, begins to operate on the human body with the utmost regularity between the hours of 5 and 7 p.m., increases in power up to midnight, wanes towards morning, and, finally, ceases to act between 9 and 10 a.m. ? A correct answer to this would be a step towards the solution of this strange problem ; only a step however, for the method of its operating would still remain to be explained."

MANSON then proceeds to discuss the influence of atmosphere, temperature and pressure, but dismisses them as having no possible effect. Periodicity bears no relation whatever to hours of sunshine, cloud or rain or other conditions influencing the quantity or kind of rays impinging directly on the human body. With terrestrial magnetism the case is quite otherwise ; its variations are rhythmical. If authorities on the diurnal variations of the declination and inclination of the compass and the intensity of terrestrial magnetism be consulted, it will be found that there is a marvellous correspondence between the rhythm of these phenomena and that of filarial periodicity. He then inserts a chart of the main daily variations of the magnetic needle taken from *Electricity* by FERGUSON, and by means of this graph, shows that the minimum of daily change of terrestrial magnetic intensity is between the hours of 10 and 11 a.m. and the maximum between 4 and 7 p.m., varying slightly with the season of the year. These hours correspond very closely with those of the commencing rest and activity of the filariae in the normal state of the body. Although these may seem wild and unjustifiable speculations, MANSON decided that they were worth considering, and activated by these speculations he made one or two unsuccessful experiments with electricity, but he was of the opinion that some expert in physiology and electricity should take the matter up. Finally he tried the effects of all kinds of drugs upon the periodicity and found them to be without effect ; these included amyl nitrate, santonin, turpentine and quassia.

On pp. 268-269 is found the table recording the effect of cold and the absence of light on filarial periodicity, whilst on pp. 355-361 there is a record of experiments (with chart extending over a period of fourteen days) demonstrating the reversal of periodicity, according to the plan of Dr. STEPHEN MACKENZIE of turning day into night and *vice versa* and showing that he was able to confirm this important observation (Dec., 1881).

On pp. 367-373, in a letter to the *Lancet*, the puzzle of periodicity is pursued still further and his closely followed arguments may well be studied with interest by students of this subject, even at the present day. As regards the actual

filaria itself, he says "periodicity may be explained by one of two suppositions: (a) the parent worm empties her uterus of mature embryos once every 24 hours, parturition going on from late in the afternoon till midnight, the young filariae live for but a few hours in the blood and are then disintegrated, as Dr. MYERS [of Formosa] has suggested, (b) parturition is a more or less continuous process—the young being nearly constantly carried along the thoracic duct into the blood. In this fluid they live for an indefinite time, circulating with it under ordinary circumstances during the night, but from some unknown cause and after some unknown fashion becoming fixed during the day." Against the assumption of "intermitting quotidian reproduction" he cites facts such as the finding of the embryos of filaria constantly during the hours of day and night in chylous urine, as well as in the lymph which constantly oozed from a lymph serotum in which he first demonstrated the adult female *Filaria bancrofti*. "The hypothesis of quotidian intermitting reproduction implies but a very short life to the embryos, but it is difficult to understand what object favourable to the host or parasite could be served by this enormous daily mortality; Nature's object in making these creatures so prolific is evidently to provide as many chances as possible for the continuation of the species. Nor is it easy to understand why other animalcules which can live for so many days out of the body should die after so short a life in it.

"The facts I have stated and other evidence . . . lead me to believe that the second hypothesis is the correct one, viz. that reproduction is continuous and that the embryos are fixed in some organ or tissue during the day. . . . Filarial periodicity may be only a pathological curio; I cannot but think, however, that it is in some way bound up with the many rhythmical phenomena, physiological and pathological, which have hitherto defied explanation. To these it may some day prove the key."

Pp. 501-503 constitute, from a historical standpoint, the most important narrative in the book, for here are recorded under the date of 10th August, 1877, the first inklings of the true nature of insect-borne disease. The narrative records that Huito (his Chinese servant) "brought me four mosquitoes which he had caught this morning in his mosquito net and which were distended with his blood. I examined them this morning." The next pages are taken up with minute details, the morphology of the embryos which he found in the stomachs of these insects and the state of the cells in the blood clot, but for the main details of the metamorphosis of the filariae in the thorax of the mosquito we have to turn back to pp. 378-415, where no less than 79 batches of mosquitoes were dissected and a record of his findings tabulated. The dates are from September to the end of October, 1883, and are the basis of the observations which he recorded in his historical paper on "The Metamorphosis of the *Filaria Sanquinis Hominis* in the Mosquito" (*Trans. Linnean Soc.* 1884, Zoology ii, 367-388). Together with these observations were found the original drawings of the various stages of the filaria in the mosquito, now exhibited in the Museum of the London

School of Hygiene and Tropical Medicine. It is interesting on looking through these scattered notes, to find that the species of mosquito in which developing filariae were found was invariably the brown mosquito (*Culex fatigans*) and that apparently 11th September, 1883, is a historic date, for it was on that day that he found in a mosquito an almost fully developed larval filaria with its characteristically lobed tail. On 14th September and subsequent dates he records the dissection of large tiger mosquitoes. These were evidently *Aedes*, and no development of the filariae was observed in these insects—which of course agrees with subsequent experience.

From 26th September onwards, he kept the insects in an incubator with temperature ranging from 85° to 87° F. and noticed that the development was much more rapid than in the previous months. It is curious that though he records the fully developed form measuring 1/20 inch in length, with a trilobed tail, yet he was never fortunate enough to discover these creatures in the neighbourhood of the head or proboscis. Yet at the same time, as pointed out in the *Life of Sir Patrick Manson*, a reviewer (possibly COBBOLD) in the *Veterinarian* for March, 1883, i.e. before the publication of the work we are considering, first suggested that the fully grown larva is deposited by the mosquito in the act of biting. This significant criticism appears to have been noted by MANSON, who treasured a cutting containing this passage in a special book he kept for this purpose, and he therefore must have read it at the time. But, as is well known, it was not until sixteen years afterwards, when ROSS had shown how the malaria parasites are injected by the mosquito, that it was again suggested by the younger BANCROFT, in the *Journal of the Proceedings of the Royal Society of New South Wales*, 1899 (p. 62).

These are the chief observations on the natural history of filariasis contained in the Diary; there are others which are mainly clinical.

On pp. 119-122 may be found a detailed account of the operative procedures in lymph scrotum and elephantiasis under the date of 17th May, 1880, together with fuller accounts of the behaviour of the microfilariae before and after the removal of the affected tissue. This, it was proved, did not produce any alteration in the numbers of embryos in the blood. In this particular case, a year after the successful operation, the man returned with elephantiasis of his right leg, which had developed in consequence of the cicatrix resulting from the operation.

Pp. 254-255 recorded the discovery of the adult *Filaria bancrofti* in the tissues of a lymph scrotum, under the date of 11th October, 1880, together with minute measurements of the worm itself and the lengths of its various organs in fractions of an inch.

On p. 272 (7th January, 1881) is a second record of the discovery of this parasite; this time in an abscess of the thigh caused by the death of the parent worm. In this only fragments of the adult worm were demonstrated but by means of these he was able to confirm BANCROFT's original discovery under similar circumstances in 1876.

On pp. 494-500 and again 505-507 is found a statement, under the date of 12th August, 1879, of his condensed views upon the pathology of filariasis and the whole question of the meaning of parasitology. This is a careful and cautious letter in answer to a request from a Dr. H. LEISRINK of Hamburg, dated 29th June of that year, asking for information on the etiology of elephantiasis for a book he was compiling with Professor ESMARCK of Kiel.

Pp. 169-179 are occupied with his observations upon the filariae of birds, and illustrated by careful comparative drawings and measurements of the parasites he discovered. Here he observed the spicule which this embryo extruded from its anterior extremity and speculated upon the use of this organ. The various "haematozoa" were found in the crow (*Corvus torquatus*), the magpie (*Pica media*), as well as in the crowned pigeon (*Goura coronata*). A list is given of many birds that he examined, including larks, kites and sparrows.

Pp. 461-491 record some interesting events of the years 1890-91 after MANSON had left China and settled down at 21, Queen Anne Street in London. These are occupied by columns of figures relating to the critical examination of blood specimens from the Congo, Nigeria, Sierra Leone, South Africa, Cochin China and Samoa, supplied by his many friends and correspondents. Here are given the data upon which the descriptions of embryos of *Filaria loa* and *Filaria perstans* were subsequently based, whilst there is a correspondence, with the original letters, with Professor LEUCKART of Leipzig, 2nd July, 1891, upon the correct nomenclature of these parasites. Here also is mentioned the embryo of *Filaria volvulus* for the first time. On p. 515 we find an account of the examination of the blood of 49 Dahomeyans who were performing at the Crystal Palace on 28th May, 1893.

This does not by any means exhaust the interesting entries in MANSON'S Diary. On pp. 204-205 are found the first references to sprue in a letter from Dr. ROWELL of Singapore, where we find it stated that there is no cure for this disease except an early departure for Europe. These were the notes which were subsequently expanded into the historic description of this disease which was published in the *Medical Reports of the China Imperial Maritime Customs*,* 1880.

Further information on this disease with reference to its dietary, are to be found in pp. 433-438.

On p. 225 there is a photograph, in a good state of preservation, of a Chinese woman with large keloid tumours of the lobules of both ears, resulting from piercing the ears for the wearing of ear-rings. It is stated that these tumours had been ten years in forming.

Pp. 275-278 contain an account of interesting experiments made with the injection of pilocarpine into the arm of an anaesthetic case of leprosy in order to test the sweating areas, under the date of 23rd February, 1881.

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On p. 279 are described investigations into trichiniasis in pigs and rats in which 219 specimens of pork were examined over a period of six weeks with the discovery of trichina parasites in two. A "puppy dog," a few weeks old, when fed upon a small amount of this material, died of acute trichiniasis.

Pp. 283-287 record an epidemic amongst fowls, muscovy ducks and rabbits (19th March, 1881). In these observations two-hourly records of body temperatures of the affected birds were kept, and it was ascertained that the virus of the disease was present in the blood stream and could be transmitted by inoculation of heart blood from one fowl to another, as well as to pigeons and rabbits, with fatal results. It is possible that he was dealing at this time with filtrable virus of fowl plague.

Skin diseases are dealt with on pp. 294-304, especially pityriasis versicolor nigra, and from pp. 57-64 are to be found the historical description of the fungus of tinea imbricata and also tinea circinata, with numerous drawings which were eventually published in 1878. References to other skin diseases will be found under scleroderma on p. 188.

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The reader who has travelled thus far successfully through this account of original work and thought will doubtless have missed some reference to malaria. But this fever and the search for the causative parasite appear to have been constantly in MANSON's thoughts. On pp. 44-49 there are some scattered notes upon some "fool experiments" as he called them, in which blood from malaria patients was placed in water at 100° F. and contrasted with similar quantities of the blood of three fowls taken at the same time. The upshot of the experiments went to show that, although the temperature of the fowl's blood rapidly declined, that of the human malaria blood did not. Six years later—in May, 1884—he returned to these experiments and attempted to cultivate the germ of malaria blood in infusions of rotting vegetable matter which he obtained from Happy Valley in Hongkong and which he boiled for two hours and then filtered. The filtrate was inoculated under strictly aseptic precautions with malaria blood, his own blood being used as a control. He reached no conclusions from these experiments, but it demonstrated the trend of his mind at that time and his intention to link the cause of malaria in some way with decaying marsh vegetation.

On p. 289, 25th March, 1881, is the record of his search of malaria blood with a magnification of 750 diameters, for the "bacillus" or microphyte of malaria, but again without any result.

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obtained from the stools which demonstrates his ability at this time as an artist, for the creature is easily recognizable as the larva of *Calliphora*. Beriberi, a disease in which MANSON exhibited an abiding interest, is mentioned only on pp. 447-449, where a well-written account is given of that disease as observed in Hongkong. Reference to the skin disease which MANSON described as pemphigus contagiosus is to be found on pp. 458-460. The contents of the vesicles was mixed with sterile water and inoculated into the skin of a volunteer, who in due course developed the lesions (July, 1887).

A milk-borne outbreak of typhoid is described on pp. 508-510. The disease affected twelve persons in whom it produced the typical fever, and a footnote to the account adds significantly "all these had milk from the same man and not from the Dairy Co."

There are many other entries in this voluminous diary which are of less general interest than those referred to above. It is possible that future research will reveal further secrets in this well-filled store. In many places where the handwriting is not particularly legible, deciphering becomes a matter of difficulty. It is hoped, however, that the fragmentary account here given will stimulate visitors to MANSON HOUSE to probe into this bible of tropical medicine, for in so doing they will discover many other references of scientific and historical interest.

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LOYAL ADDRESS SENT TO
HIS MAJESTY KING GEORGE V.

ON THE OCCASION OF HIS SILVER JUBILEE, MAY 6TH, 1935,

BY THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

To
The
King's Most Excellent Majesty

WE the Fellows of the Royal Society of Tropical Medicine and Hygiene hereby convey to Your Majesty our loyal and dutiful greetings on the historic occasion of Your Majesty's JUBILEE. The ties of fealty and affection that unite the subjects in this Realm to the Throne are forged the stronger by the knowledge of Your Majesty's ever-present concern in all that touches the health of the people. This Society has for its aim the advancement of the science, and the extension of the domain, of TROPICAL MEDICINE, and your Majesty and His Royal Highness the Prince of Wales have signalized the Royal commendation of our labours by the gracious acceptance of the offices of Patron and Vice-Patron, recognition which serves as a continual encouragement in our unrelenting endeavour.

On the successful issue of the truceless strife against tropical disease depend not only the health but the very existence of millions of our fellow subjects in Your Majesty's possessions beyond the seas and Your Majesty's reign is already distinguished by discoveries in tropical medicine which will result in the saving of a great multitude of lives. Thus the maladies which heretofore have exacted so heavy a tribute of human life in the tropic zone, one by one yield up their secrets to science and are brought under control, and the travail and devotion of a host of workers will bring to pass still greater triumphs within the compass of the years which we pray God will grant Your Majesty to reign over us.

Signed on behalf of the Fellows of the Royal Society of Tropical Medicine and Hygiene

Leonard Rogers

President

C. M. Wemyss

N. Hamilton Fairley

Honorary Secretaries

Manson House
Portland Place
London

MAY 6TH. 1935

OBITUARY.

WALTER GAWEN KING, C.I.E., M.B., C.M., D.P.H.,
COLONEL I.M.S.

1851-1935.

Colonel W. G. KING was born 4th December, 1851 and joined the I.M.S. on the 31st March, 1874.

Arriving in India on the 31st October of that year, he was sent to Madras Presidency where he spent the greater part of his long service. After being in charge of various Madras Infantry and other regiments he was appointed Surgeon to the Duke of Buckingham in 1876; shortly after, he became Professor of Physical Science, Presidency College, Madras, and in 1882 Professor of Hygiene in the Medical College, Madras. In 1890, he was appointed Inspector of Vaccination; and, 2 years later, Sanitary Commissioner, Madras, a position which he filled with great distinction until 1905.

During these years Colonel KING impressed the authorities with his work in the famines of 1876 and 1896, studied the epidemiology of cholera and built up the first Public Health Department in the Indian Empire. His proposals for the reorganisation of the Sanitary and Vaccination Departments of the Madras Presidency were made in connection with the resolution of the first Indian Medical Congress held at Calcutta in 1894. His scheme contemplated the establishment of a vaccine institute with a bacteriological superintendent, and the employment of well-qualified sanitary and vaccination staffs in proportion to the population of the areas concerned.

A special course of training in the Medical College, Madras, was instituted; and the first class assembled in January, 1895. From that date a constant flow of trained sanitary inspectors was made available to the Public Health Department of the Presidency. Also, as a result of Colonel KING's proposals, the *Madras Registration of Births and Deaths Act* of 1899 was passed.

From 1891 Colonel KING repeatedly urged the importance of the Madras Presidency possessing a central vaccine institute where high grade animal lymph

could be cultivated and preserved under conditions which would guarantee the requisite standard of purity and activity, and he advocated the advisability of uniting a bacteriological laboratory with this institute. This received the Government's consent in 1899 : building operations were commenced in 1901 and by May next year the institute which was given the name of the " King Institute of Preventive Medicine " was completed. In this way the Government made gracious acknowledgment of the valuable services rendered by Colonel W. G. KING, Sanitary Commissioner, to the people of the Madras Presidency in matters of public health.

In September, 1905, Colonel KING was transferred to Burma as Inspector-General of Civil Hospitals and Sanitary Commissioner, where he completed his service.

He retired in 1910, but constantly maintained an active interest in all public health questions and specially in regard to vaccination and the problems associated with the preparation and distribution of an active vaccine lymph. During the War he wrote a memorandum on smallpox ; and from 1914 to 1922 he was a member of the Council of the Royal Society of Tropical Medicine and Hygiene.

Colonel KING was without doubt the father of public health in South India and on the occasion of the unveiling of his portrait in the office of the Director of Public Health in March, 1930, the Minister in charge of Public Health rightly referred to him as the original founder of preventive medicine in the Madras Presidency.

His death on 4th April, 1935, at the ripe age of 83 years removed a very prominent member of the Indian Medical Service, and one who for many years had taken an active interest in this Society.

A. J. H. RUSSELL.

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE
AND HYGIENE.

VOL. XXIX. No. 2. JULY, 1935.

Proceedings of the 28th Annual General Meeting of the Society held at
Manson House, on Thursday, 20th June, 1935,
at 8.15 p.m.

Major-General Sir LEONARD ROGERS, K.C.S.I., C.I.E., F.R.S.,
President, in the Chair.

BUSINESS.

REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1935.

The PRESIDENT presented the Annual Report ; copies of which had been circulated.

Dr. F. Murgatroyd proposed the adoption of the Report. The motion was seconded by Major J. A. Cruickshank, and carried unanimously.

REPORT OF THE HON. TREASURER FOR THE YEAR ENDED
31ST MARCH, 1935.

Sir Arthur Bagshawe, *Hon. Treasurer*, in presenting his Report said :—
As I have to appear shortly in another rôle, I shall not occupy much of your time now. We have had a very successful year, finishing with a balance of £476 on the ordinary Income and Expenditure Account.

The debt on Manson House has been reduced by £960, leaving £13,745 still to raise. Of this amount £13,000 is borrowed on mortgage and the remaining £745 will probably be paid off in the present financial year.

Dr. V. B. Wigglesworth : I am sure we are all highly gratified at the satisfactory state of affairs which the Treasurer's Report reveals, and I have great pleasure in proposing the adoption of the Report.

This was seconded by Dr. C. R. Amies and carried unanimously.

ELECTION OF AUDIT COMMITTEE.

Dr. R. D. Mackenzie proposed that Dr. V. S. Hodson, Dr. R. P. Garrow and Major J. A. Cruickshank be elected members of the Audit Committee. This was seconded by Professor Warrington Yorke and carried unanimously.

RESULT OF BALLOT FOR PRESIDENT, TWO VICE-PRESIDENTS AND
COUNCILLORS FOR 1935-37.

Dr. N. Hamilton Fairley, *Hon. Secretary*, announced the Result of the Ballot as follows :—

President :

Sir ARTHUR BAGSHAW, *C.M.G.*, M.B., B.Ch., D.P.H.

Vice-Presidents :

Sir JOHN MEGAW, *K.C.I.E.*, D.Sc., M.B., B.Ch., Maj.-Gen. I.M.S.

Professor D. B. BLACKLOCK, M.D., D.P.H., D.T.M.

Councillors :

P. A. BUXTON, M.R.C.S., L.R.C.P., D.T.M. & H., Professor.

Sir S. RICKARD CHRISTOPHERS, *C.I.E.*, *O.B.E.*, F.R.S., Col. I.M.S. (ret.).

S. F. DUDLEY, *O.B.E.*, M.D., F.R.C.P., Surg.-Capt., R.N.

N. HAMILTON FAIRLEY, *O.B.E.*, M.D., D.Sc., F.R.C.P.

E. D. W. GREIG, *C.I.E.*, M.D., D.Sc., F.R.C.P.E., Lt.-Col. I.M.S. (ret.).

A. E. HAMERTON, *C.M.G.*, *D.S.O.*, Colonel, late R.A.M.C. (ret.).

S. P. JAMES, *C.M.G.*, M.D., F.R.S., Lt.-Col. I.M.S. (ret.).

WILLIAM W. JAMESON, M.D., F.R.C.P., Barrister-at-Law, Professor.

W. P. MAC ARTHUR, *D.S.O.*, *O.B.E.*, K.H.P., M.D., F.R.C.P.I., Col., late R.A.M.C.

J. W. SCOTT MACFIE, D.Sc., M.B., Ch.B., D.T.M.

F. P. MACKIE, *C.S.I.*, *O.B.E.*, M.D., F.R.C.P., F.R.C.S., Bt.-Col. I.M.S. (ret.).

OSWALD MARRIOTT, M.D., M.R.C.P.

A. J. R. O'BRIEN, *C.M.G.*, *M.C.*, M.B., Ch.B., M.R.C.P., D.P.H.

H. HAROLD SCOTT, M.D., F.R.C.P., F.R.S.E., D.T.M. & H.

Sir THOMAS STANTON, *K.C.M.G.*, M.D., F.R.C.P., D.P.H., D.T.M. & H.

J. GORDON THOMSON, M.B., Ch.B., M.R.C.P., Professor.

Sir MALCOLM WATSON, M.D., D.P.H.

C. M. WENYON, *C.M.G.*, *C.B.E.*, M.B., B.Sc., F.R.S.

F. NORMAN WHITE, *C.I.E.*, M.D., D.P.H., Major I.M.S. (ret.).

H. E. WHITTINGHAM, *C.B.E.*, F.R.C.P.E., D.P.H., Group-Capt. R.A.F.

The President (Sir LEONARD ROGERS) : Fellows and ladies and gentlemen, it is customary for the retiring President to say something about the activities of the previous years. Fortunately it is not necessary to take much time over that, because our Society is happy in having no recent history other than that of steady progress in our work, in the reading of papers, the organising of discussions, and the publishing of results in other papers, which go all over the Empire, and far beyond it. Last year I was glad to see that the sale of our TRANSACTIONS and of reprints, and advertisements brought in nearly £500, which is much higher than previously, showing how much our publications are appreciated. We may safely say that the discussions and papers in the last two years have been up to our usual standard. The credit of that is largely due, in the first place, to the senior Hon. Secretary, Dr. WENYON, whose work has been most ably seconded by the junior Hon. Secretary, Dr. HAMILTON FAIRLEY.

You have heard, from the Treasurer's Report, that our finances are in a flourishing condition. During the last year we have paid off nearly £1,000 of the debt on Manson House, and that has been largely due to our having a legacy of £500, for which we are indebted to our energetic Secretary, Miss Wenyon. The result is that there now remains a debt of only £13,745. As our building and our furniture are valued for insurance purposes at nearly £30,000, we are in a very satisfactory position, and I wish to give credit to the previous Council, under the presidency of Dr. CARMICHAEL LOW, for the courageous manner in which they carried through the great scheme in regard to MANSON HOUSE in the time of the country's serious financial depression. The greatest credit is due to them for that.

Progress has been made, too, in connection with the Library, and it has been decided to bind regularly a further twelve of the most important journals. Colonel CRAWFORD, of the I.M.S., has presented, through me, to the Society a valuable collection of books by I.M.S. officers, and others, which he has collected in the last forty years. You will be able to see them in a new book-case : in another new book-case we are placing the journals which have been bound. I wish to draw your attention to the fact that there are certain missing numbers, which we shall be glad if Fellows can supply in order to complete our series of volumes. Dr. H. H. SCOTT was appointed Honorary Librarian this year. He has taken great trouble with the Library, and some books which were obviously useless have been weeded out.

The Society has lost during my term of office a distinguished Past President, Major-General Sir HAVELOCK CHARLES ; and we have had to record the death also of two Honorary Fellows, Dr. THEOBALD SMITH and Dr. FRIEDRICH FÜLLEBORN.

INDUCTION OF THE NEW PRESIDENT.

Our new President, Sir ARTHUR BAGSHAWE, is so well known to you that I need not say much about him. He worked in Uganda from 1900 to 1907, and was the first to discover the pupa of the tsetse fly. His paper describing this was buried in the archives of the Royal Society, which is the best place I know for burying information, so it is not as well known as it deserves to be. In 1908, BAGSHAWE was home on leave, and he was asked to take on the Sleeping Sickness Bureau, and that led to the Tropical Diseases Bureau. That work, though not "showy," is most valuable. I might mention my own case. When I retired from India I gave the whole of my journals to the School of Tropical Medicine, Calcutta, except my set of the *Tropical Diseases Bulletin* which I would not part with: that is the only one I kept! Sir ARTHUR BAGSHAWE is one of those quiet men who work so efficiently that it makes their work appear easy, though in reality, it may be very difficult. I have the greatest confidence in handing over the presidency to him, which I will now proceed to do without wasting any more of your time.

Sir ARTHUR BAGSHAWE was then invested with the President's Badge and Chain of Office.

The President, Sir Arthur Bagshawe: Sir LEONARD ROGERS, ladies and gentlemen, I feel very greatly honoured by being elected President of this Society, and I thank you, Sir, for the kind things which you have said about me and my work. My association with this Society goes back a long way, rather more than 25 years, and as I was looking at the names of past presidents I realised that I have seen twelve of them inducted. I cannot be absolutely sure I was present on each occasion, but I think I was. I was not present when Sir PATRICK MANSON first took the chair: presumably he inducted himself.

It is a very proud thing to be one of a line of such distinguished men, the names of some of whom will live as long as the world lasts, one may almost say. I thank you very much for electing me, and I shall do my very best to maintain the honour and usefulness of the Society.

Appointment of Vice-President.

By the Laws of the Society, the incoming President elects one of the vice-presidents. I have chosen as my Vice-President Dr. HORNER ANDREWS, of the Ministry of Agriculture. We have usually had a veterinarian on our Council: at present we have not one. I think you will agree that Dr. HORNER ANDREWS is a very suitable representative of the veterinary branch of our profession.

PRESENTATION OF MANSON MEDAL.

My first duty as President is a very pleasant one, and that is to present the MANSON MEDAL. It is awarded every third year to the author of such original work on any branch of Tropical Medicine or Hygiene as the Council may consider to be deserving of the honour. On this occasion the Council has selected as the recipient our former President, Prof. J. W. W. STEPHENS, F.R.S., of Liverpool University. I think you will all agree that the selection is a very good one for the name of STEPHENS is known throughout the world in connection with pioneer work in tropical medicine, particularly malaria, the study of which he commenced many years ago when he was a member of the Royal Society's Commission in Central Africa. He has contributed largely to precise knowledge of this disease including blackwater fever and the methods of quinine administration. He first identified *Plasmodium ovale* as a new malarial parasite of man, and has suggested under the name *Plasmodium tenue* that still another form exists. He was jointly responsible for the establishment of *Trypanosoma rhodesiense* as distinct from *Trypanosoma gambiense* as the cause of an acute form of human trypanosomiasis. These are but a few of Professor STEPHENS' contributions to our knowledge of tropical medicine, to the successful study of which over a wide field he has devoted the greater part of his life. It is, therefore, with great pleasure that I present to Professor STEPHENS the highest award of our Society, the MANSON MEDAL.

Professor J. W. W. Stephens : Sir, I have the great pleasure of being the first to address you in your presidential capacity. We are told, in our Year Book that the Manson Fund of 1921 was raised with the idea of revivifying the esteem in which MANSON was held by his friends and his admirers. The occasion on which that was done was the removal of the old London School of Tropical Medicine, of which he was the creative spirit, from its old home in the Royal Albert Dock. And to-night on this occasion, I think that the Society not only takes the opportunity of honouring one of its Fellows, but also desires to revivify and keep green the name of MANSON. All the same, the name of MANSON would not die, even if this Society and Manson House were to cease to exist, for the name of MANSON is inseparably linked with the origin of Tropical Medicine as a special branch of study.

It is with some apprehension I have noticed that I have now become the sole living recipient of the MANSON MEDAL, and I have been wondering what the four old medallists will say when I have the pleasure of joining them in the Elysian fields. They might say, "Who is this Fellow?" not in any opprobrious sense, but Fellow of the Royal Society of Tropical Medicine! I think, however, I should be welcomed by them, for I have tried, as we all do, to add something to the sum total of our knowledge which, sooner or later, will gain a complete victory over tropical disease. Sir, I have to thank Sir RICKARD CHRISTOPHERS and Colonel MACKIE, my proposer and seconder, and the Society for the honour they have conferred upon me, and it is with grateful and very sincere feelings that I do so. ♣

PRESENTATION OF CHALMERS MEDAL.

The President (SIR ARTHUR BAGSHAWE): We have yet another medal to present, the CHALMERS GOLD MEDAL. It was founded in 1921, by Mrs. ALBERT CHALMERS who I am glad to see is with us this evening, in memory of her husband, the late Dr. ALBERT CHALMERS, the author of many important contributions to tropical medicine. This award is made biennially to a worker under forty-five years of age in recognition of research of outstanding merit contributing to our knowledge of Tropical Medicine and Hygiene. On this occasion the medal has been awarded to Professor WILLIAM H. TALIAFERRO, of the University of Chicago, who is distinguished for his researches into the immunology of protozoal and helminthic infections. Of special merit was his demonstration of ablastin, a new type of immune body, in *Trypanosoma lewisi* infections, the introduction of a skin test for filariasis, and the demonstration that increased phagocytic power of a hypertrophied reticulo-endothelial system is the basis of immunity in malaria. In a very important monograph he has reviewed the whole subject of immunity to infections with animal parasites.

Unfortunately, Professor TALIAFERRO is unable to be present this evening. He has asked Dr. WENYON to receive the medal on his behalf. I will, therefore, ask Dr. WENYON to receive the CHALMERS MEDAL and transmit it to Professor TALIAFERRO in due course.

Dr. Wenyon, in receiving the medal, said that Professor TALIAFERRO had asked him to express his deep appreciation of the honour which the Society had conferred on him.

Proceedings of an Ordinary Meeting of the Society held (after the Annual General Meeting) at Manson House, 26, Portland Place, at 8.45 p.m., on Thursday, 20th June, 1935.

Sir ARTHUR BAGSHAWE, C.M.G., M.B., D.P.H., *President*, in the Chair.

DEMONSTRATION.

TEMPERATURE CHARTS ILLUSTRATING THE ACTION OF
ATEBRIN MUSONATE INTRAMUSCULARLY COMPARED
WITH QUININE BIHYDROCHLORIDE INTRAMUSCULARLY
IN THE TREATMENT OF MALARIA IN CEYLON.

BY

S. SOMASUNDRAM, M.R.C.P. (LOND.), D.T.M.

Physician, Kandy Hospital, Ceylon.

When Dr. BRIERCLIFFE, Director of Medical and Sanitary Services, Ceylon, knew that I was coming to England he suggested it might be of interest to Fellows of this Society if I showed them some of the charts which I have exhibited this evening. These, as you will have seen, have to do with the treatment of malaria in Ceylon during the present epidemic of which you have all heard, and the action of the new drug atebirin musonate. First of all, however, I will make a few remarks about the usual treatment with atebirin by oral administration.

Atebrin tablets by mouth have been extensively used in Ceylon for the treatment of malaria in hospital patients, the standard course being one tablet 0.1 gramme, t.d.s. p.c. for five days. Patients who were given atebirin on admission took on an average at least 3 days for the temperature to come to normal. Patients who were given quinine bisulphate $7\frac{1}{2}$ grains in mixture t.d.s. p.c. took on an average about $1\frac{1}{2}$ to 2 days for the temperature to come to normal.

In those that did not react properly to the drug employed, a change of treatment, *i.e.* from atebirin to quinine or from quinine to atebirin produced very favourable results.

With regard to subsequent attacks of fever, a 5 days' course of atebirin was found to keep patients free from symptoms of malaria for a much longer period than did the short courses of about 5 to 7 days of quinine which are customary in Ceylon. Patients returning with fever after a course of atebirin were found

to be more seriously ill, while it took longer to bring their temperature to normal.

Toxic effects of atebtrin are not uncommon. Atebrin by the mouth frequently caused epigastric pain which may be severe especially if it was given on an empty stomach. Symptoms of mental derangement also occurred in a fair number of cases either towards the end of a course of treatment or shortly after its completion. They usually lasted two to three days. Discolouration of the skin has been very rare and when it occurred was usually limited to the palms and soles. In one case of malaria with chronic nephritis death occurred after two days of treatment, *i.e.* after six tablets had been taken. In children atebtrin by the mouth produced vomiting and diarrhoea.

Atebrin musonate is a soluble salt of atebtrin. It is a bright yellow powder supplied in glass ampoules. Each ampoule contains 0.125 grammes of atebtrin musonate which is equivalent to 0.1 gramme of atebtrin, the adult dose being the contents of three such ampoules which are readily soluble in 10 c.c. of cold water. It can be given either intravenously or intramuscularly. In Ceylon the preliminary tests with this drug were made in January this year by Dr. SIMEONS of Bombay who claimed that one intramuscular injection of 0.375 gramme would effect a cure of malaria. It was, however, found on further experience, that one injection was insufficient either to control the temperature or to free the blood from parasites. Thereafter two intramuscular injections were given, each of 0.375 gramme at an interval of 24 hours. This, as will be seen from the charts shown this evening, brought the temperature down in the majority of cases and caused the disappearance of the asexual stages of the parasites (whether *P. vivax* or *P. falciparum*) as a rule within three days. In a few cases it was necessary to give three injections. Ill nourished children were liable to collapse shortly after the injection, with vomiting, giddiness and fainting.

The charts shown are those of unselected cases treated at the Kandy Hospital, Ceylon, with two intramuscular injections of atebtrin musonate and, for purposes of control and comparison, similar cases treated at the same time with two intramuscular injections of one gramme each of quinine bihydrochlor., followed by quinine bisulphate $7\frac{1}{2}$ grains in mixture by mouth, t.d.s. p.c.

I have shown you the two series of charts in order that you may draw your own conclusions on the relative merits of the two methods of treatment. I myself have not been able to arrive at the conclusion that treatment with atebtrin musonate is in any way superior to that of quinine, as regards the immediate effects. It is not possible to make any statement regarding the relative frequency of relapses after the two treatments for, quite apart from the fact that there has been insufficient time to make reliable observations, the patients treated are immediately exposed to reinfection when they return home.

I have also drawn your attention to the toxic effects which have sometimes followed atebtrin injections and which do not occur with quinine. It should be noted, however, that the atebtrin musonate intramuscular injections are quite painless in which respect they are superior to those of quinine.

PAPERS.

TYPHUS FEVERS IN THE TROPICS.

BY

MAJOR-GENERAL SIR JOHN MEGAW, K.C.I.E., D.Sc., M.B., I.M.S.

I find "this typhus business" rather perplexing, and in some ways rather tantalizing. Fifteen years ago I could have told you something new and important about the subject, whereas now that the typhus fevers have become fashionable, my personal experience is at least two or three years out of date, and that is a long time in connection with a disease group which is being so ardently studied all over the world. Typhus fevers are not merely in the fashion, they have also acquired a considerable entertainment value, even with the lay public. For example, *Rats, Lice and History*, by HANS ZINSSER, has become a "best seller," and deservedly so.

Let me say a word or two about the name "typhus." You may be inclined to ask, What's in a name? ; but there is a great deal in the name typhus. Much of the confusion which surrounds the typhus fevers is due to the names which have been applied to them. Many of these names convey misleading suggestions, and even the medical profession is very much influenced by suggestions. The word "typhus" is a very old one ; it was applied originally to almost any kind of acute fever which tended to appear in epidemic form and was accompanied by severe nervous symptoms. The term "typhoid state" was probably more closely connected with typhus fever than with typhoid fever. About a hundred years ago typhoid fever was discovered to be a separate entity, so was relapsing

fever, and therefore the name "typhus" came to be applied to severe acute cases of contagious fever of ten to twenty days duration, and accompanied by a peculiar skin rash. The disease was obviously associated with filth, overcrowding and malnutrition. Until 25 years ago matters remained in much the same state. Then, almost simultaneously, two great discoveries were made: one, that typhus fever was transmitted by lice; the other, that peculiar bodies, later known as *Rickettsia*, were present in the vector insects and in the tissues of people suffering from the disease. The word "typhus" thus came to be applied to a definite disease entity, and everything appeared to be simple. But even 25 years ago two other diseases of a similar type were known: the spotted fever of the Rocky Mountains, and the Japanese river fever. They were recognised by many people to have a close clinical resemblance to typhus fever, and a few years later they also were found to be associated with rickettsia bodies. But their epidemiological features were in striking contrast with those of classical typhus fever, and so the diseases were regarded as distinct entities. SAMBON, it is true, insisted that spotted fever of the Rocky Mountains was the same as typhus fever, but his views were not accepted. There was also another fever, Brill's disease, which BRILL described, nearly 40 years ago, as a new disease. BRILL recognised a similarity, in many respects, to mild typhus fever, but he thought it impossible that a disease occurring in a sporadic way, obviously not communicated from man to man, and mild in type, could have anything to do with typhus fever. Later it was found that this disease gave the same serological reactions as typhus, and that there was a cross-immunity between Brill's disease and typhus fever. Accordingly, by 1912, this disease came to be looked upon as an inter-epidemic form of typhus fever. A few years ago Brill's disease came to be regarded as endemic typhus, a disease of rats conveyed to man by fleas. Now, even more recently, opinion has veered round once more: ZINSSER has pointed out that about 97 per cent. of the recent cases of Brill's disease in New York occurred in immigrants from countries where typhus fever occurs. His view is that cases of Brill's disease are flares-up of a typhus infection which had been lying dormant in the persons concerned. Brill's disease is a very good example of how names change their meaning from time to time. The disease has been the same for centuries, but the name "Brill's disease" has had four different meanings within a comparatively short time.

Between 1910 and 1915 several workers described fevers which had obvious points of resemblance to typhus. SMITHSON in Queensland, CONOR and BRUCH in Tunisia, MACNAUGHT in South Africa, McKECHNIE in an unpublished work in India, and SCHÜFFNER in Sumatra described cases which bore a clinical resemblance to typhus, but were so different in their epidemiological features that none of their sponsors, except McKECHNIE, suggested that they were forms of typhus.

I have been personally interested in this disease since 1916, when I had an attack of a fever resembling typhus; this had almost certainly been conveyed

to me by a jungle tick in the Himalayan forests. I described the fever in the *Indian Medical Gazette* in January, 1917, and suggested that my case was one of Brill's disease, and that the fevers described by the workers whom I have just mentioned should be classed as a sub-group of typhus fever. Having received reports of similar cases from various people in India, including Major-General SPRAWSON and Colonel CHAPMAN, and having made a further study of the subject, I wrote a note in the *Indian Medical Gazette* of October, 1921, in which I expressed the following conclusion: "my personal experience and a consideration of the available evidence have led me to form a strong suspicion that the disease is one affecting the animals of the jungle and that it is conveyed to man by a tick, and that the disease is either the same as Rocky Mountain fever, or at any rate is closely related to it. The disease is probably widely distributed in India and other parts of the world, but remains unrecognised because of its superficial resemblance to typhoid fever." In my note I proposed the following nomenclature:—

"*Provisional Classification of the Fevers of the Typhus Group.*—

(I) Louse Typhus; (II) Tick Typhus; (III) Mite Typhus."

The paper was well abstracted in the *Tropical Diseases Bulletin* of January, 1922, but, apart from that, very little further attention was attracted to the typhus-like fevers until Dr. WILLIAM FLETCHER, from 1926 onwards, wrote his well-known papers on tropical typhus. Those papers not only threw a flood of light on the subject, but attracted the attention of the medical profession in such a way that since that time there has been a continuous stream of literature on the typhus fevers. Dr. FLETCHER was very much struck by the resemblance between the cases which he saw and those which I had described as Indian tick typhus. But there were some puzzling differences: FLETCHER could find no evidence that his patients had been bitten by ticks; he found that the agglutination responses were of two kinds in his cases, and both were different from those observed in Indian tick typhus. Gradually the differences were explained: one kind of tropical typhus turned out to be mite typhus, the other has recently been identified with flea typhus. This flea typhus is the latest accession to the group of typhus fevers, and its exact position is still somewhat doubtful. It gives similar serological reactions to louse typhus and clinically the cases are similar to those of mild typhus. But in a paper which came to me two days ago from Dr. PIJPER of South Africa, who has done a good deal of careful work on the subject, it is shown that flea typhus does not protect against louse typhus, though the latter protects against flea typhus. ZINSSER, on the other hand, says that rats harbour the virus of classical typhus, that this is transmitted from rat to rat by fleas or by lice, that it can be transmitted from rat to man by infected rat fleas, and then from man to man by human lice. Later in his book he says the rat virus soon reverts to its original type, and does not continue to be transmissible from man to man by lice. This is a point which needs to be cleared up. I do not know of any clear evidence that rat typhus in natural

conditions does change to the form of human typhus which is communicated from man to man by lice, though MOOSER, of Mexico, seems to suggest that such an event is of every-day occurrence. Can it be that louse typhus and flea typhus co-exist in Mexico? I express no opinion on this subject, but so far as tick typhus and mite typhus are concerned, they have been known for many years, and I have not heard of any case in which the virus of tick typhus or mite typhus has been modified, in natural conditions, so as to behave like the virus of louse typhus. Many cases of the former two diseases have been recorded : in some of these there must have been opportunities for lice to become infected from the patients, and so to originate an epidemic of louse typhus. But I have not heard of a single instance in which there has been a suspicion of any such occurrence. Therefore, so far as mite and tick forms of typhus are concerned, they differ from louse typhus in that patients suffering from them can be treated in open hospital wards without any obvious risk of the disease being communicated to other people. If the virus of flea typhus is the same as the virus of louse typhus, differing only in a temporary lowering of virulence, then there may be a risk of an outbreak of louse typhus originating from a person suffering from flea typhus. I hope someone will be able to throw light on this very important point.

To return to the name "typhus," there has been, all along, a surprising reluctance to use the word typhus to designate the fevers which we are discussing, in spite of the fact that they are very like typhus in their clinical features and pathology. The fact that their epidemiology is so different seems to have kept people from admitting the possibility of their belonging to the same group. This prejudice is at last breaking down, and the invaluable *Tropical Diseases Bulletin* has set the seal of its approval on the suggestion that they are forms of typhus fever : it has recently classed them as "The Typhus Group of Fevers," instead of "Typhus and Unclassed Fevers."

Some people go to the other extreme and say that these fevers are obviously typhus ; why not call them typhus and have done with it? In other words, why give them qualifying names? I do not agree with that attitude, because these non-epidemic typhus fevers differ so much from the epidemic typhus in their mode of transmission, and consequently in the methods of prevention, that they deserve a special name. Tick, mite and flea typhus differ from each other in certain important respects, but the crucial difference lies between epidemic typhus which is transmitted directly from man to man and the non-epidemic fevers of the typhus group which are conveyed from an infected animal to man by arthropods.

The latter are thoroughly deserving of special names, at any rate in the cases in which the vector can be determined. There are some cases in which it is impossible to say which vector is concerned, but we hope that the work of Dr. FELIX and others on the serological reactions will help us in doubtful cases, because there seems to be growing evidence that each kind of typhus

shows its own special serological response to the various types of *Proteus* organisms. If it turns out that each virus is specially associated with one distinct arthropod vector, the provisional classification which I suggested in 1921 may take a permanent place.

The nomenclature of these fevers is really a very important matter. In opening a discussion a year ago I gave a list of 25 names, mostly misleading, which had been applied in recent years to the non-epidemic typhus fevers. I will not repeat them now, but I am sorry to see that the process of coining new names is still being continued. The latest names are even worse than the earlier ones: look at the name "eruptive Mediterranean fever"! I need not bring forward arguments in condemnation of that name, you can see how inappropriate it is. Another is "dothiendermic aiguë," I hope we shall not have to employ such a tongue twister. The use of the name of the vector as a distinguishing name for these diseases is open to criticism. In certain cases we are not sure what the vector is, and we have not yet complete proof that the same virus may not be communicated by two or more vectors. When I originally suggested the names "louse typhus," "mite typhus" and "tick typhus" I would hardly have dared to put forward the proposal, but for the encouragement I received from Col. W. F. HARVEY, then Director of the Central Research Institute, Kasauli, with whom I had discussed the problem. In the cases in which there is no clear evidence as to which vector is concerned, we have to fall back on the name "non-epidemic typhus fever of unknown vector."

Just as there has been much confusion over the names of the fevers, so also there have been differences of opinion with regard to the names of the associated rickettsia bodies. I must regretfully disclaim the honour which has been done me by AMARAL and MONTEIRO, who have proposed to call the virus of "tropical typhus" by the name *Rickettsia megawi*: the name is quite inappropriate. General HARVEY has suggested a nomenclature which appeals more to me; it is simpler, and corresponds closely to my provisional classification of the disease. He suggests *Rickettsia orientalis* for mite-typhus, *Rickettsia rickettsi* for the tick typhus, and *Rickettsia prowazeki* for the virus of louse and flea typhus. I disagree only in thinking that the virus of flea typhus is deserving of a separate name, because it differs rather definitely from the virus of louse typhus, perhaps the name *Rickettsia murina* or *R. fletcheri* would be suitable for the virus of flea typhus. So far as students and practitioners are concerned, they may accept as a safe working hypothesis that there are four different kinds of fever which have the right to be regarded as members of the typhus group. Each tends to conform to a fairly characteristic disease type, the only difficulty being that sometimes "inapparent" forms of them occur in which there is no rash and very little fever, but these are not of great practical importance, as they do not seem to be transmissible from man to man, and are never fatal so that a mistake in diagnosis will do no harm to anybody and in any case will rarely be detected. All these four fevers are associated with, and probably caused by,

rickettsia bodies, each fever appears to be transmitted by its own special arthropod: the louse-borne fever is conveyed directly from man to man, and can rightly be called epidemic typhus, while the others are conveyed from an animal reservoir to man by fleas, ticks, and mites respectively, and so they are non-epidemic, sporadic, place diseases.

Speculations as to the remote ancestry of the viruses are of great interest, but not of much importance. Experiments for the purpose of discovering the behaviour of the virus in the lower animals are of interest and importance, but the really essential thing for the doctor to know is, how these fevers behave in Nature, how to diagnose, manage and prevent them.

TYPHUS FEVERS IN MALAYA.

BY

WILLIAM FLETCHER, M.D., F.R.C.P

Sir JOHN MEGAW's paper is of special interest because he was one of the first to draw attention to non-louse-borne typhus, and to realize that typhus exanthematicus is not a disease which stands alone, but is one of a large group of related fevers.

The non-louse-borne diseases of the typhus group have come into greater prominence during the last few years because they have spread in places where they were formerly unknown, and as they have spread they have sometimes become more deadly. In the Malay States, tropical typhus was first recognized in 1924, and with an intimate knowledge of the country dating from 1903, I can vouch that it was a new disease. When I left, in 1927, it was not dangerous to life, but by 1931, in which year 220 cases were diagnosed in the laboratory alone, the mortality had increased to 12 per cent., and the death of several healthy young Europeans had alarmed the public. It has been suggested that the mite-borne virus is carried from country to country by migrating birds in the larval mites which often cluster in bunches beneath their feathers (the clusters of larval mites in a rat's ear or on a bird's breast, are so small that they look like little patches of scurf or saw-dust stuck to the skin); the flea-borne virus may be carried over the sea in grain and merchandise as plague is carried.

One of the most important recent developments in the study of typhus is the discovery of the virus in the brains of rats in practically every country where a careful search has been made. Rodents have been known for many years as the carriers of the tsutsugamushi disease, and in 1931 MOOSER, CASTENEDA and ZINSSER discovered the virus of Mexican typhus in the brains of wild rats caught in a gaol where several cases of typhus had occurred among the prisoners. The rickettsial viruses of the typhus group of diseases are carried from rodents to man by larval mites, ticks and fleas. In ticks, the virus can pass from generation to generation and from tick to tick without the intervention of a vertebrate host. In mites, only the larva is a blood-sucker and, as it feeds only once, the virus must be passed on through the nymph, the adult and the egg to the next generation before it can be transmitted to another host. The adult mite feeds only on vegetable juices. It is possible that rickettsiae were originally parasites of the plants which form the food of adult mites, and that the earliest typhus-like diseases were those carried by mites or ticks. The classical typhus exanthematicus probably appeared much later, when men took to living in caves and perhaps acquired their lice from the bats with which they shared them.

The position of murine typhus resembles that of murine leptospirosis with regard to the multiplicity of strains; the work of LEWTHWAITE shows that there are numerous typhus viruses in the rats of the Malayan jungle, where he has recovered strains which differ antigenetically not only from human strains, but also from one another. The wide diffusion of typhus virus in rats, all over the world, raises the important question whether the murine virus can give rise to epidemics of classical typhus. Can a person who has become infected with

endemic typhus, carried by rat-fleas, originate an epidemic of classical louse-borne typhus, if he should happen to have lice in his clothes? MOOSER implies that this happens in Mexico, and he has shown that lice can be infected with the virus of endemic typhus by intracoelemic inoculation. But the epidemiology of the disease makes it appear unlikely that the flea-borne murine virus of endemic typhus is continually, under ordinary circumstances, originating outbreaks of louse-borne epidemic typhus, though this may happen among a population of lowered resistance in close contact with rats.

The minute bodies known as *Rickettsia* which are found in all the diseases of the typhus group appear to constitute the virus. Virus-vaccines prepared from the rickettsiae of infected arthropods have been in use for some years. A potent protective vaccine is prepared in the Rocky Mountain Fever Laboratory, at Hamilton, Montana, from ticks infected with *Rickettsia rickettsi*, and hundreds of people are inoculated with it every year. A vaccine prepared from *Rickettsia prowazeki* is employed for the protection of persons who are specially exposed to infection with typhus exanthematicus. It is prepared from the intestines of lice infected by Weigl's method of rectal injection, and a skilled worker can inoculate a thousand lice in a day—a number sufficient for the vaccination of between 60 and 70 persons. In Mexico, a vaccine prepared from mammalian rickettsiae is employed. ZINSSER and CASTENEDA have found that if rats are first exposed to X-rays, and then inoculated with the virus of endemic typhus, enormous numbers of rickettsiae appear in the peritoneal exudate. These bodies, washed free from extraneous substances, killed with formalin and used as a vaccine, give protection against the virus, and produce a positive Weil-Felix reaction after inoculation. This vaccine has also been employed for the production of an immune serum in horses.

As regards the classification of the typhus diseases it seems that as FELIX has already suggested the most satisfactory method is primarily serological and secondarily according to vector. A serological test can be applied with ease in each individual case but a proved vector can very rarely be found. When typhus first appeared in the Malay States it did so as a group of cases clinically indistinguishable from one another, and it was the Weil-Felix reaction which first showed us that we were dealing with two diseases, differing in their immunity reactions, their epidemiology and their vectors. A simple classification in accordance with the Weil-Felix reaction alone is not sufficient, for example, the diseases due to the virus corresponding to *Proteus* X19 (*i.e.* endemic and epidemic typhus) must be divided into flea-borne and louse-borne.

FELIX and RHODES have shown that identity of Weil-Felix reactions accompanies cross immunity, but the mystery of the connexion of *Proteus* X strains with the rickettsial viruses of the typhus group remains unsolved. Strains of *Proteus* XK, which is agglutinated by the blood of people suffering from the rural form of Malayan typhus, have been isolated from several patients suffering from this disease in the Malay States, but they do not behave as a true virus. Dr. FELIX is here himself and will doubtless have something to say about this.

THE SEROLOGY OF THE TYPHUS GROUP OF DISEASES.

BY

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We have listened to two most lucid reviews of the history and of the present knowledge of the diseases of the typhus group. To make the picture complete one particular aspect of the problem needs to be added, *viz.*: the serology of these diseases. I shall discuss it very briefly. All those present, I assume, are regular readers of the *Tropical Diseases Bulletin* and are well aware of the fact that what was generally presented for many years as "Typhus and Unclassed Fevers" has recently been transformed into "The Typhus Group of Fevers." Sir JOHN MEGAW, who was a pioneer of this conception, has already mentioned this fact. I should like to add, that immunological methods have played the decisive rôle in the process by which this transformation was accomplished.

In our knowledge of the serology of the typhus group of diseases a turning point was marked by FLETCHER and LESSLAR (1925, 1926) in their studies on tropical typhus in the Federated Malay States. This work for the first time established the existence of two distinct serological types of typhus fever. As soon as this work was confirmed and amplified (FELIX and RHODES, 1931), two far-reaching conclusions had to be drawn: (1) that cases of typhus-like diseases could no longer be excluded from the typhus group on the basis of a negative agglutination reaction with *Proteus* X19, and (2) that negative results of cross-immunity tests between viruses differing in their reactions to *Proteus* X did not necessarily imply complete diversity of origin. Cross-protection could not be expected between viruses which, by their relationship to various serological types of *Proteus*, were shown to be different antigenically.

The close correlation between cross-immunity and agglutination reactions with various types of *Proteus* X is illustrated in Table I. The Table, which was published in the *TRANSACTIONS* of this Society (FELIX, 1933) demonstrates the results only of those experiments on cross-immunity where the test virus employed has been the virus of classical louse-borne typhus from Eastern Europe or Tunis.

Table I shows that cross-protection has been obtained only with those viruses which possess the same main (or major) antigen as that of classical typhus virus, namely the O antigen of type X19. On the other hand, the viruses of Rocky Mountain spotted fever and "fièvre boutonneuse" yielding negative cross-immunity tests do not possess main antigens of this type. It is seen from the Table that in the latter two diseases the agglutination results with type X19, X2, and XK are recorded as group reactions. The remarkable fact thus appears to have been well established, that cross-immunity between different varieties of typhus virus is accompanied by identity of the serum

reactions to *Proteus* X, while failure to obtain cross-protection coincides with dissimilarity in agglutination reactions.

Table I also shows that the results of cross-immunity tests between different viruses of the typhus group can be predicted from the agglutination reactions obtained with various types of *Proteus* X. The same, of course, is also true *vice versa*. I have made such "prophecies" on several occasions and so far they have not yet proved me a false prophet. It must be stated, however, that it is not always possible to determine from the agglutination reactions of the patients' serum whether an antigenic factor is to be classed as main or group

TABLE I.
CROSS-IMMUNITY TESTS AND AGGLUTINATION REACTIONS WITH *PROTEUS* X TYPES.

Name of Disease.	Locality.	Vector.	Agglutination with <i>Proteus</i> X Types.			Type of Main Antigen of Virus.	Cross-Immunity Tests with Classical Typhus Virus.
			X19	X2	XK		
Classical typhus	Eastern Europe and Tunis	Lice	+++	+	—	OX19	Positive
Tabardillo	Mexico	Lice and rat fleas	+++	?	—	OX19	Positive
Endemic typhus (Brill's disease)	South Eastern U.S.A.	Rat fleas	+++	?	—	OX19	Positive
Fièvre nautique	Toulon (War-ships)	Rat fleas	+++	?	—	OX19	Positive
Rocky Mountain spotted fever	Western U.S.A.	Ticks	+	+	+	Unknown	Negative
" Fièvre boutonneuse "	Marseilles, Tunis	Ticks	+	+	—	Unknown	Negative

+++ = main agglutination. + = group agglutination. ? = not tested adequately.

antigen. For this purpose the most reliable test is that for the production of agglutinins in rabbits, by infecting the animals with the virus under test. The combination of cross-immunity experiments in the guineapig with tests for agglutinin production in the rabbit was, therefore, recommended for the analysis of the antigenic structure of different typhus viruses (FELIX, 1933, b). This procedure has recently been followed in typhus research in various parts of the world and to my knowledge the results so far obtained have in no

instance been inconsistent with the thesis of the intimate relationship between cross-immunity and serum reactions to *Proteus* X.

During the last few years the serum reactions of different varieties of typhus have been investigated by numerous workers. The following table was published two years ago (FELIX, 1933) showing the agglutination reactions of the serum of patients suffering from these diseases, so far as they were known at that time. Though the table, according to present knowledge, already requires some alterations and additions, it is nevertheless reproduced unaltered, in order better to serve the purposes of the present discussion.

The agglutination reactions recorded in Table II are specified as main and group agglutination and it is seen that of the three types of *Proteus* X now generally used, only two, *viz.* X19 and XK have so far been established as the main antigenic types of typhus viruses. The third type, X2, is of special importance in the tick-borne varieties of typhus, though the question whether it is to be considered as a major or minor antigenic factor is still under investigation (PIJPER and DAU, 1935 ; DAVIS, 1935).

The general impression is conveyed by Table II that the different members of the typhus group of diseases would perhaps fall into the same sub-groups whether they are classed according to the transmitting vector or according to the antigenic type of the virus. And this leads to the important question of classification which Sir JOHN MEGAW has discussed.

If there were that close correlation between vector and antigenic type of the virus, which seems to be suggested by Table II, the classification of these diseases would be a simple matter. However, this is not the case. The table, although published only two years ago, is no longer up to date and recent investigations suggest that the correlation between mode of transmission and antigenic type of the vector is apparent rather than real.

Firstly, there is the well-established case of the flea-borne endemic typhus of world-wide distribution. Its virus is antigenically identical with that of the louse-borne variety. More recently PIJPER and DAU (1934, 1935) have made very interesting observations on three different varieties of typhus occurring in South Africa, namely: (a) an epidemic form, occurring in louse-infested patients in Basutoland; (b) a sporadic form, met with in certain towns and associated with the rat-flea; (c) the variety known as tick-bite fever of South Africa, which is prevalent in the open country. PIJPER and DAU succeeded in isolating the viruses of all three varieties and studied their behaviour in cross-protection experiments in guineapigs and in tests for agglutinin production in rabbits. All three types of virus appear to be closely related if not identical in antigenic structure and this result is in full agreement with the observations on the agglutination reactions of the patients suffering from the three varieties of the disease. If the work of PIJPER and DAU is confirmed, and I have no doubt it will be, we shall be confronted with the fact, that South African louse-typhus and South African flea-typhus are not identical with similar diseases in other

TABLE II.
MAIN AND GROUP AGGLUTININS IN DIFFERENT VARIETIES OF TYPHUS.

Name of Disease.	Locality.	Vector.	Agglutination with <i>Proteus</i> X Types.			Type of Main Antigen of Virus.
			OX19	OX2	OXK	
Classical typhus	Old and New Worlds	Lice	+++	+	—	OX19
Tabardillo	Mexico	Lice and rat fleas	+++	?	—	OX19
Endemic typhus (Brill's disease)	South Eastern United States	Rat fleas	+++	?	—	OX19
Fièvre nautique	Toulon (Warships)	"	+++	?	—	OX19
Endemic typhus	Australia	Unknown	+++	+	—	OX19
Tropical typhus, type W (Shop typhus)	Malaya and Dutch East Indies	"	+++	+	—	OX19
Tropical typhus, type K (Scrub typhus)	"	"	—	—	+++	OXK
Tsutsugamushi	Sumatra and Malaya	Mites	—	—	+++	OXK
"	Japan	"	—	—	+++ ?	OXK ?
Rocky Mountain spotted fever	Western and Eastern United States	Ticks	+	+	+	Unknown
" Fièvre boutonneuse "	Marseilles, Tunis	"	+	+	—	"
" " " (Febbre eruttiva)	Italy	"	+	+	+	"
Tick-bite fever	South Africa	"	+	+	+	"
Tick-typhus	British India	"	?	?	?	"
Sao Paulo endemic typhus	Brazil	"	+++	+	+	OX19

+++ = main agglutination. + = group agglutination. ? = not tested adequately.

parts of the world. Finally, there are some recent observations from India indicating that what used to be called the tick-typhus of India includes a variety of serologically different types of typhus.

In view of these facts it seems quite obvious that the typhus group of fevers cannot be classified according to the transmitting vector. The earlier suggestion by Sir JOHN MEGAW (1921) to classify the then undetermined typhus-like fevers as members of one group of diseases, was undoubtedly an attempt in the right

TABLE III.
TYPHUS GROUP OF FEVERS.

Sub-group.	Type X19.	Type XK.	Type Undetermined.
Name of disease	CLASSICAL EPIDEMIC TYPHUS Tabardillo Endemic typhus (Brill's) of U.S.A. and Australia, Greece, Syria, Manchuria, Malaya (shop typhus) and Toulon (fièvre nautique)	JAPANESE RIVER FEVER (Tsutsugamushi fever of Japan, Malaya and Dutch East Indies) Malay scrub typhus Scrub typhus of East Indies	SPOTTED FEVER OF ROCKY MOUNTAINS Sao Paulo endemic typhus Fièvre boutonneuse Febbre errutiva Tick bite fever of S. Africa Epidemic and endemic typhus of S. Africa India tick typhus
Vector	Lice and rat fleas	Mites	Ticks, lice and rat fleas
Reservoir of virus	Rats Man	Field mice and rats	Rodents Dogs ? Ticks Man
Agglutination	X19 +++ X2 + XK —	X19 — X2 — XK +++	X19 + X2 + XK +

direction and so long as the group was composed only of a few diseases the differentiation according to vector seemed to fit the known facts. This, however, is no longer the case.

In a recent number of the *Journal of the Royal Army Medical Corps* (March, 1935), the classification of the typhus group, as given in Table III above, has been suggested. I share the responsibility for this suggestion with Colonel J. HEATLEY-SPENCER.

Table III shows that this classification is based on the antigenic type of the virus, on which immunity and immunity reactions depend, and thus follows

a principle generally accepted in microbiology. At present it is a simple scheme, comprising only three sub-groups. With further growth in our knowledge of these viruses this provisional scheme will, of course, grow more complicated. Although published only a few months ago the table has already had to be brought up to date by inserting the results of the recent work from South Africa. It seems to me that the suggested scheme is a correct presentation of our present knowledge of these viruses and provides an adequate framework for inclusion of results of future work.

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DISCUSSION.

Major-General D. Harvey: As Sectional Editor for the Typhus Group of Fevers in the *Tropical Diseases Bulletin* I have the opportunity of studying the various papers sent to me. One point Sir JOHN MEGAW mentioned, that is the question of Brill's disease in New York. ZINSSER and his co-workers have recently shown that the virus isolated from cases of Brill's disease in New York is the human or classical old world typhus virus, whereas the virus of endemic typhus in America and elsewhere is the murine or rat virus; if this is so it will be necessary to separate endemic typhus from Brill's disease. If the virus of Brill's disease, which is an endemic sporadic disease, is the classical virus then this virus may be both epidemic and endemic. Brill's disease is not carried by lice nor according to ZINSSER has the rat-flea, rat, or tick anything to do with it, but man himself is the reservoir of the disease. ZINSSER believes that these cases of Brill's disease in New York are recrudescences of an infection originally contracted in Europe, some 97 per cent. of the cases investigated occurred in immigrants from countries in Europe where typhus is or used to be epidemic, such as Poland and Russia; some of these people have been resident in New York for from 10 to 30 years; the disease does not spread to contacts born in America.

Sir JOHN mentioned recent work on typhus in South Africa; apparently the South African viruses of louse-borne, tick-borne and flea-borne typhus have their own peculiarities; for instance in South Africa there is not complete cross immunity between the epidemic and endemic viruses, whereas in other countries a definite cross immunity has been proved. There are also serological differences. Apparently the typhus viruses differ in different countries.

Recently I had a talk with one of our officers just home from India and he

considers that mite-borne typhus exists in Southern India and is a severe form of the disease.

There is also the question of the identity or non-identity of the epidemic or human virus and the endemic or rat virus.

Professor NICOLLE of Tunis is the leader of the "dualists," he considers that although the viruses are closely related they are not identical and cannot readily be changed the one into the other. MOOSER and others consider that a case of endemic typhus (rat virus) may be the starting point of an epidemic of louse-borne typhus under certain circumstances. But this question is one of many still unanswered.

Colonel J. Heatly-Spencer : I think that a great deal of what I was going to say has been much more ably said by Dr. FLETCHER, Sir JOHN MEGAW and Dr. FELIX, but I will say a word or two about the difficulties of classification.

The greatest credit is due to Sir JOHN MEGAW for his original classification on the vector basis. That sufficed so long as we only knew typhus fever in its three main divisions: epidemic typhus, Rocky Mountain typhus or fever, and Japanese River fever. But it is unfortunate that this original vector classification has been extended to try and cover this complicated group of diseases which we know now as the typhus group. In particular, I think the term "tick typhus," of India, has ceased to be a useful one, and is rather confusing because, under this heading, typhus fevers are reported in which not only do the antigenic properties of the virus vary very much, but in which there is, in most cases, only presumptive evidence of tick bite. Recently I looked through a series of cases reported under this heading, and I could only find something less than 1 in 5 in which there was evidence of tick bite. We cannot exclude the possibility of other vectors, particularly in those which show a preponderance of agglutinins to *Proteus* XK. In some of the recent cases reported as tick typhus from India I have seen some with a preponderance of agglutinins to X19, others to XK. A few weeks ago I was told of groups in India showing agglutinins or agglutinin preponderance to X2. Further details of these cases will be given in two papers of importance to be published shortly.

There is a similar difficulty in adopting a clinical classification. Dr. FLETCHER has said he thought that the *Proteus* X19 group ought to be further classified as lice and flea spread groups. The classical group might be classified on a clinical basis as epidemic, endemic, and recrudescent, the last to cover the modern conception of Brill's disease.

With regard to the other groups and sub-groups, no clinical classification seems possible. We have to come back to the facts available, and they are easily demonstrable. The facts are the agglutinating properties to *B. proteus* strains.

In particular I wish to identify myself with Dr. FELIX, particularly from the point of view of those who have to lecture to students. You will have noticed the complexity of some of Dr. FELIX's earlier tables, and when you have to instil their contents into a class of students it becomes a very disheartening thing.

Major-General Sir John Megaw (in reply): I will confine my further remarks to one or two points as it is much too late for an exhaustive reply to all the matters referred to by the speakers.

In Table II in Dr. FELIX's paper, you may have noticed that when he was referring to the tick typhus of India, he put a query mark in the column of agglutination response. When I was studying that disease, the only *Proteus* organism we had was *Proteus* X19 from Kasauli: the response to this was negative except for an occasional reaction of 1 in 80; a high titre agglutination was never observed.

In reply to Colonel SPENCER, I freely admit that the classification proposed by me is provisional, but those who have, quite properly, criticised my nomenclature have not suggested an alternative. If they did, I think their classification would be open to more objection than mine. In India, the name tick typhus has come into such universal use that every case of non-epidemic typhus is usually assumed to be one of tick typhus even when there is no evidence to to incriminate the tick.

Colonel SPENCER suggests the use of the name "endemic typhus" instead of "non-epidemic typhus." A year ago, at the discussion in Bournemouth I expressed a preference for the name "endemic," but unfortunately the word has been ear-marked for flea-typhus. Another possible name "sporadic" typhus is hardly suitable as louse-borne typhus is often sporadic.

General HARVEY criticizes the use of the term "epidemic" as a designation of louse-typhus, as sporadic cases often occur: the criticism is justified, and I have only fallen back on the name for want of a better; and, after all, other diseases are called epidemic although they commonly occur as sporadic cases. What I want specially to suggest to the student is to get down to something very simple. I suggest that he should fix his mind specially on the two main groups, the epidemic and the non-epidemic typhus fevers; in other words, louse-borne typhus, and the non-louse-borne forms of typhus which are transmitted from animal reservoirs to man.

Colonel SPENCER suggests that there are several kinds of typhus in India; I agree, and I shall be surprised if we do not find both flea-borne and mite-borne as well as tick-borne typhus. But I think most of the cases reported hitherto have been tick typhus. Colonel SPENCER raised the point that the tick has been found only about once in every five cases. I am prepared to accept those figures; indeed, I should have said once in every seven or eight cases. When the tick is found it has nearly always attached itself in some situation where it is protected from friction by the clothes. In my own case it was attached above a prominent collar bone. In others it has been in such sites as the external auditory meatus, the umbilicus, among the suprapubic hairs or on the scrotum. The suggestion is that in most cases the tick feeds and finding its surroundings irksome drops off; only when protected from disturbance does it remain attached for any length of time. As the bite is usually quite painless the tick often escapes detection, so that failure to find the tick is not evidence justifying the exclusion of the tick as a possible or even probable vector.

COMMUNICATIONS.

AVITAMINOSIS B2.

BY

J. V. LANDOR

AND

R. A. PALLISTER,*

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INTRODUCTION.

GOLDBERGER and WHEELER (1920) in their pioneer experiments on pellagra gave eleven volunteer convicts a deficient diet and produced a dermatitis of the scrotum in six of them, a dermatitis upon the dorsum of the hands in two cases, and an erythema of the neck in one. This they accepted as being experimentally produced pellagra in human beings and they considered that probably there was missing from the food a specific factor mainly to blame for the occurrence of pellagra, and this they designated the PP (pellagra preventive) factor.

GOLDBERGER and LILLIE (1926) having found that dried yeast contained the factor that would seem to prevent pellagra in human beings and also experimental black tongue in dogs, subjected such yeast to heat for 2½ hours in the autoclave at a pressure of 15 pounds, and found that it would then still prevent experimental black tongue but would no longer prevent polyneuritis in dogs. They also found that they could produce a pellagra-like dermatitis in rats which they could prevent and cure by adding autoclaved yeast to the

*We are indebted to many of our colleagues for assistance in this investigation and in particular we wish to thank Dr. ABDUL SAMAT, Dr. K. DAMODARAN and Dr. H. C. SAMUEL for the supervision of the cases at Singapore and Johore. We wish to acknowledge, too, our gratitude to Prof. J. L. ROSEDALE, College of Medicine, Singapore, for help and advice.

For permission to publish we are indebted to Dr. R. D. FITZGERALD, Director of Medical Service, Straits Settlement, and Dr. G. H. GARLICK, Principal Medical Officer, Johore.

diet which would produce it. They concluded that the factor concerned was the PP factor, and that it was the chief if not the only factor concerned in the causation of pellagra, of experimental black tongue in dogs, and of their experimental dermatitis in rats.

The Biochemical Society of England about a year later adopted the nomenclature of vitamins B₁ and B₂ for the anti-neuritic and anti-dermatitic portions of the water-soluble B vitamin, while in America SHERMAN and AXTMAYER (1927) described them by the letters F and G. While admitting that this nomenclature, and even the whole subdivision of the vitamin B complex, is still not entirely satisfactory, we have adopted it for the purposes of this paper in the absence of any better.

The disease that is to be described in this paper as avitaminosis B₂ has manifested itself by two sets of symptoms. It may be that two diseases are really present but we believe that the symptoms form an early and a late stage of one condition. Our reasons for adopting this view will be given later. The early stages of the disease are characterised by epithelial tissue changes. An eczematous condition of the scrotum and a sore mouth are the main lesions. The tongue is affected with superficial glossitis, and the angles of the lips become white and cracked. In the later stages there are nerve symptoms suggestive of sub-acute combined degeneration of the cord but nearly always accompanied by dimness of vision.

In various tropical countries symptoms similar to these have been described. JENNER WRIGHT (1930) describes as the "A and B avitaminosis disease of Sierra Leone" a disease characterised by lesions of the mucous membranes and skin, especially at the muco-cutaneous junctions, associated with or followed by disorders of the nervous system, and curable by the addition of cod liver oil and yeast to the diet. He found that subcutaneous injections of 2 c.c. of radiostoleum daily in addition cleared up obstinate stomatitis cases in a week. There is no reference in this pamphlet to any attempts to cure the disease by yeast alone. STANNUS (1930) has long held the view that such lesions are pellagrous and in discussing the literature, particularly with reference to Africa, shows how widespread such a condition is. LUCIUS NICHOLLS (1933) describes a disease occurring in Ceylon prisons the characteristics of which are "a papular dry skin eruption frequently accompanied by a mild neuritis and (or) eye symptoms such as night blindness, dimness of sight, xerophthalmia or keratomalacia." These patients are very liable to diarrhoea and dysentery. He has given the disease the name phrynoderma because the "toad skin" is the commonest symptom. He attributes the disease in the main to vitamin A deficiency. Later he published an account (NICHOLLS, 1934) of further investigations into the incidence of vitamin A deficiency among school children and other members of the population. His standards of examination were dry papular skin and sore mouth and he found these lesions were widespread. We wrote suggesting a wider deficiency might be present and mentioned our

experiences on B deficiency in Malaya. We think, however, with him and others (PILLAT, 1929; LOEWENTHAL, 1933) that a papular eruption on an abnormally dry skin is probably a manifestation of a deficiency condition of which vitamin A is the chief ingredient at fault. The lesion is very rare among the patients we are about to describe and our experience of it is limited.

FITZGERALD (1932) describes an outbreak in an Assam prison of exfoliative glossitis with ulceration at the angles of the mouth, with diarrhoea but with no affection of the serotum. He ascribes it to three factors, (i) excess of condiments in the diet, (ii) weakness of the intestinal tract from previous illness such as dysentery and (iii) slight deficiency of vitamin B₂ in the diet. On removing chillies from the diet, 70 per cent. showed improvement or cure (but this result was without controls); and on adding one ounce of yeast to the diet the figure was improved to over 90 per cent. Cod liver oil he found of no value. His paper stimulated us to investigate whether irritant condiments played any part in our disease but we have been unable to find any evidence to that effect. MOORE describes a syndrome in adolescent schoolboys, in an institution in West Africa, of dimness of vision, sore tongue and mouth, and itching of the serotum which got better when the boys went home for holidays and so away from the institution diet. He came to the conclusion that the disease was due to lack of vitamin B₂, and we think his syndrome must be very nearly identical with ours. In a recent paper, however (MOORE, 1934), he blames particular foods also, such as "gari" and "kassava" in Africa, especially for the dimness of vision. GOODWIN (1934) has recently described a case in London with a skin lesion of the phrynoderma type but also with some moist eczema at the corners of the mouth. The possible lack of vitamin B was not eliminated, as a liberal diet was given in addition to treatment by cod liver oil. We have heard from Hongkong that the lesions of the mouth and serotum are also found in the prison there though the nerve symptoms have not been noted. It would appear therefore that symptoms of this type are widespread throughout the world.

Our attention was drawn to the condition because of its prevalence in the prisons at Singapore and Johore* that have been recently under our care. The disease is not entirely confined to institutions, but cases among the public are not common. It is not a new condition and probably is less severe now than it was some years ago. A senior member of the prison staff remembers it during the whole of his thirty years' service and he says that it used to be very troublesome. Bowel diseases and other infections are rare in the prison and, apart from the disease here described, the prisoners are very healthy: they appear to be well fed and complaints are rarely heard. Beriberi, once so serious a disease in Eastern institutions, does not now occur either in the peripheral neuritis form or in the acute cardiac form, the diet having been planned to provide a sufficiency of vitamin B₁.

*The Johore Prison referred to in this paper is the main prison of the state and is situated in the town of Johore Bahru.

The routine differs somewhat in the two prisons but it will be enough if some particulars are given of the Singapore Prison. The ordinary long sentence prisoner is given for the first few months of his detention manual labour and then if his behaviour is satisfactory he either works at his own trade or learns some useful trade such as laundering or bookbinding. He receives the lowest scale diet (C) at first. After 3 months he progresses to B diet, and after 6 months to 1 year he receives A diet, which is the best, for the remainder of his sentence. These three diets as given to Chinese (who are the most numerous race in the prison) are shown in the appendix. The diets of other nationalities are very similar in type differing only to suit custom or religion. The Johore diets differ only in minor points from the Singapore diets. The rice given at both prisons is usually of the "parboiled" variety, *i.e.*, it has been partially cooked before husking, and the husk is then removed and leaves a brownish rice grain. Such rice may contain a little vitamin B, but as it is reboiled in water before being eaten, and the cooking water often thrown away, it is doubtful whether it can be regarded as a source of vitamin B. The prisoners are given other foods containing vitamins B1 and B2, but our evidence in this paper goes to show that the supply of B2 must be deficient.

SIGNS AND SYMPTOMS.

A.—EARLY STAGE OF EPITHELIAL LESIONS.

The eczematous condition of the scrotum is the most troublesome lesion and in a well marked case the pain and irritation are almost intolerable. The scrotum may present any of the various appearances that are well known in eczema (Plate II, Fig. 6). The worst cases are those of weeping eczema, where the skin is red and moist and the exudation of fluid forms crusts. In other cases the rugae are all lost and the skin is dry, smooth, red and glazed with superficial cracks running across. In some mild cases there is merely a white branny desquamation. The different forms seen vary in different individuals and, to some extent, the appearances vary from time to time in the same patient. The whole of the skin of the scrotum is usually affected about equally and if a patchy distribution is observed it raises a suspicion that an infection of ringworm is present. The lesion does not spread on to the surrounding skin but occasionally a little scurfiness may be found around the anus. The skin at the free margin of the foreskin is white and sodden in a few cases.

If the mouth is now examined a group of lesions will usually be found here (Plate I, Figs. 1, 2 and 3). The mouth is not complained of by the prisoners to the same extent as is the scrotum. Severe lesions are less common than severe lesions of the scrotum but evidence of involvement can be observed in the majority of cases. Slight signs are extremely prevalent in the prison and are not rare among the poorer members of the general public. The tongue of the average coolie frequently presents a different appearance from that of the healthy

PLATE 1.



FIG. 1.

An early case of sore tongue. The papillae are prominent and "geographical" areas can be seen.



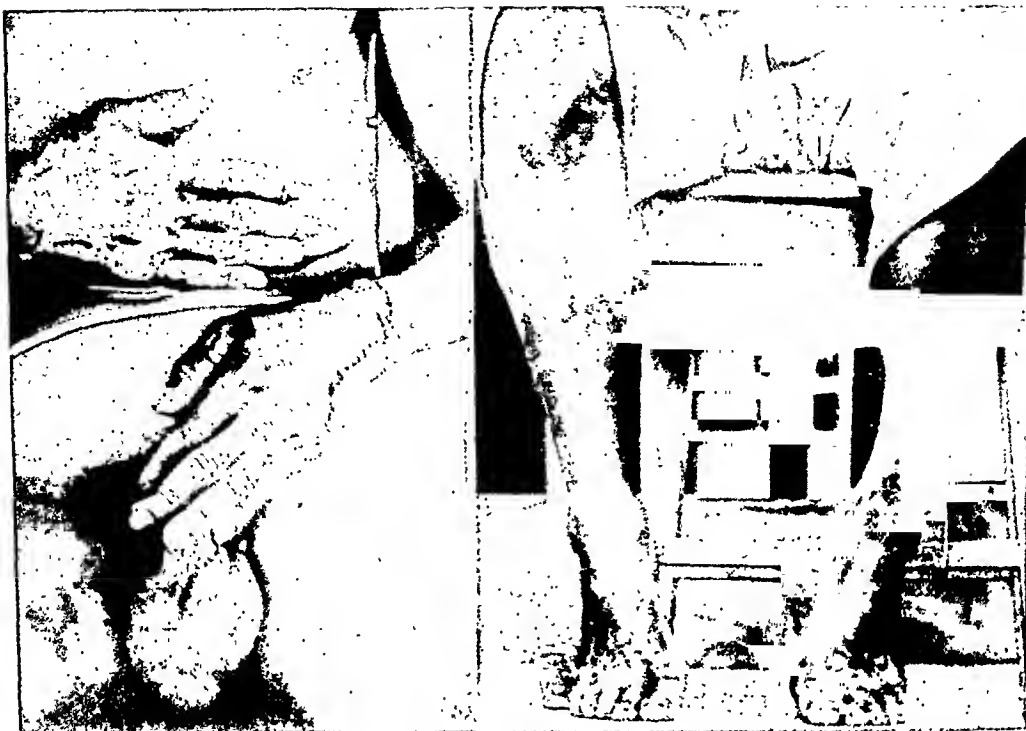
FIG. 2.

Tongue with an area of desquamated epithelium.



FIG. 3.

To show eczematous condition at the corners of the mouth.



Figs. 4 and 5. To illustrate case of pellagra referred to in text.



Fig. 6. Eczema of the scrotum.

European. It has a thin white fur and may be slightly glazed. Indentations round the margins made by the teeth are not uncommon. In the earliest stages of this disease the papillae round the tip and margins become swollen and red and stand out prominently. Areas on the surface of the tongue are then affected in a "geographical" manner. The white fur is lost and the underlying papillae are seen to be red and inflamed. As the condition progresses the whole tongue is affected. The epithelium is lost and the red sore tongue of superficial glossitis results. The final appearance is of a small red tongue quite smooth from the desquamated epithelium. Few cases, however, among the prisoners become as severe as this. The corners of the mouth are affected more or less equally with the tongue. The muco-cutaneous junction in this region becomes white and sodden with heaped-up epithelium and painful fissures may develop. Occasionally a little scurfiness may be seen on the skin around the mouth. The disease develops in different individuals at different times after admission and complaints are rarely heard until some months have passed. Nevertheless about two or three months after entering prison nearly 50 per cent. of the prisoners develop some or all of these symptoms. In the course of a prophylactic experiment 61 control cases were examined at the end of 3 months; of these 23 presented lesions of the scrotum (the stomatitis was not considered a definite enough lesion for this experiment). During the next few months there is a slight increase and some cases become so sore as to be incapable of work if untreated. The natural course of the disease appears to last a few months, for it is found that among prisoners of long standing (e.g., about 1 year or over) the percentage affected is lower, for example only 71 were found affected out of 378 such prisoners examined though many more (164) said that they had suffered from the complaint earlier. At first sight this might be put down to altered diet but this was tested by giving 20 new admissions A diet and it was found that there was no appreciable difference between this group and a control group of 20. In Johore Prison a survey was made on August 29th, 1933, and out of a total of 393 prisoners 138 were found affected with sore scrotum and stomatitis, 59 with stomatitis only and 8 with sore scrotum only. The percentage with the epithelial disease was thus 52.2, but it must be mentioned that in many cases the mouth signs were mild. In the Singapore Prison the incidence of sore mouth did not appear to be so severe as in Johore.

B.—LATE STAGE OF NERVE LESIONS.

In this stage the central nervous system is affected. The first case seen complained of pains and weakness of the legs with failing eyesight. The condition did not resemble any common nervous complaint but on investigation a number of prisoners were found more or less decrepit, with similar symptoms. The men affected had all been in prison for a long time and the onset of serious symptoms was from six months to some years after admission. Early symptoms

in this stage are alterations in sensation in the lower extremities. Complaints are made of numbness of the feet or legs or of tingling sensations. Sometimes the patients describe pains like needles being jabbed into the limbs. These symptoms always seem worse at night. In many prisoners the condition does not seem to become worse than this, but in some cases weakness and stiffness of the legs develop. About the same time complaint is made of poorness of vision which in a few of the bad cases is accompanied by mild photophobia. No complaint of night blindness has been received and on being questioned the patients say they dislike bright light most. In the majority of cases eye and leg symptoms are both present. Cases occur, however, not infrequently where only one part is affected. The examination of a moderately severe case reveals a few definite physical signs. The patient is a little unsteady especially when he is turning and there may be a resemblance to a tabetic gait. Rombergism is present in all bad cases. The knee jerks and ankle jerks are exaggerated in most cases but, as in sub-acute combined degeneration of the cord, a few cases, often those of long standing, have normal or diminished reflexes. The Babinski reflex is either absent or flexor. The sensation is diminished or absent in a "stocking" fashion to the ankles, knees or even on to the trunk. Both sides are nearly always equally affected. Very occasionally the diminution of sensation is patchy. Touch, pain, and deep sense are all affected. The cremasteric and abdominal reflexes are present. The sphincters are not affected. The lower part of the abdomen and sometimes the upper part may show some loss of sensation and even the face may be affected. The arms are affected in a manner similar to the legs but much less severely and complaints are rare. The tendon reflexes are exaggerated and sensation is lost over a "glove" area. No inco-ordination of the arms has been observed. The loss of vision varies from a haziness down to less than 6/60 on Snellen's test types. No case of complete blindness has been observed. Refraction and general physical examination do not reveal any gross changes. The most constant finding among severe cases is a slight pallor of the optic disc affecting particularly the temporal halves. In a few cases conjunctivitis or corneal ulceration, probably coincidental, have occurred but no case of xerosis conjunctivae has been seen although such cases are not very rare among the poorer Indian members of the public and their children. The pupil reacts to light and accommodation but in some cases the light reaction is poorly sustained. The condition would appear to be a retro-bulbar neuritis followed by a partial optic atrophy. Mentally some of these patients appear rather dull but it is doubtful whether there is more change than could be accounted for by the physical disabilities.

All the really definite cases of this central nervous system disease have been in prison for over 1 year and, therefore, the evidence of the disease should be measured as a percentage of prisoners of over 1 year's residence. In Singapore Prison on the 6th February, 1934, there were 1,210 prisoners of whom 461 had been resident for 1 year or over ; on this day there were 34 prisoners (all having

been in prison about 1 year or over) who appeared to be definite cases ; that is to say, about 7 per cent. of the 461 were affected. A third of these cases were severely crippled. One case only was bedridden. He had been transferred to the local hospital (as a prisoner) before we took charge of the prison and was never under our care. It is difficult to estimate the incidence accurately because minor symptoms appear to be much commoner. On the other hand some of the cases included in the 34 were not at all severe. In the Johore Prison the incidence was similar ; at the survey made on 29th August, 1933, there were 10 definite cases among 393 prisoners, *i.e.*, 2½ per cent. At another survey made a month later there were 140 prisoners who had been resident over 1 year and 9 of them had some or all of these nerve lesions, *i.e.*, 6·4 per cent. No cases of beriberi either of the wet or dry type have been observed while we have been in charge of these institutions.

It is convenient here to consider the reasons for supposing that the central nervous system disease is a late stage of the epithelial disease. A certain number of the cases of eczema of the scrotum complain of pain, tingling or numbness in the lower extremities, or of hazy vision. For example 25 cases of eczema of the scrotum were questioned and 9 said that some or all of these symptoms were present. As evidence in support of the genuineness of these subjective symptoms there is an increased knee jerk which is very commonly found. All the cases of the central nervous system disease stated that at some time during their life in prison they had suffered from eczema of the scrotum. On questioning other prisoners of similar length of residence only about two-thirds admit to having had this eczema. The work of MELLANBY (1931), however, on this subject must not be overlooked. He found that when diets containing a large amount of cereal and deficient in vitamin A are eaten by puppies, degenerative changes in the spinal cord develop. If the nervous symptoms in the disease we are describing were due to lack of vitamin A one would expect to find some evidence of the early symptoms of avitaminosis A such as dry papular skin or night blindness. These symptoms are absent and such evidence as we have points, in our opinion, to a relationship between nerve symptoms and epithelial symptoms.

ETIOLOGY AND TREATMENT.

A.—THE STAGE OF EPITHELIAL LESIONS.

The disease has been seen in Chinese, Malays and Indians. One case was in a European prisoner. Most are Chinese who form the majority of the prisoners. Apart from the prisons at Singapore and Johore where we have carried out most of the investigations, we have seen the disease in Kuala Lumpur (F.M.S.) Prison, in the Mental Hospital at Singapore, and among the boys of the Singapore Reformatory. We see occasional cases among poorer members of the public. The patients are nearly all males as they form the greater majority of the prisoners. We have seen cases of sore mouth in women

and we have no reason to suppose that women differ appreciably in their susceptibility.

Men of various trades and occupations have become affected after entering prison. The different tasks, light or heavy, and the different locations in the prison do not appear to influence the incidence of the disease. There is one exception to this, namely, prisoners who have anything to do with the cooking of food rarely get the disease, and this point will be discussed under "diet." The staff, Asiatic and European, of the prisons have not been found to suffer from this disease, though many of them may suffer from ringworm of the groin or elsewhere. We think that this rules out an infective origin. Their diet is under their own control and is not fixed as is that of the prisoners.

The previous health of the prisoners appears to have no special influence on the course of the disease. The Wassermann reactions were taken of the blood of ten cases of the disease at Johore and all happened to be negative.

We have not noted any special degree of anaemia among these patients but blood counts have been done and these show nothing worse than a slight secondary anaemia. Some of the cases of eczema of the scrotum have also tinea cruris in addition ; only a few cases showed the fungus either direct or by cultural methods, but it must be borne in mind that such methods do not always show the fungus readily in tropical ringworms.

Diet.

In the Johore Medical Report for 1931, the epithelial symptoms are mentioned as being prevalent in the Johore Prison and there was a suspicion of them being due to vitamin deficiency. This report also mentions that the disease had been investigated 15 years previously when it was thought to be due to the use of tinned-iron plates for food and it appeared to be lessened in incidence by the substitution of enamelled iron ones. Aluminium plates are now used, however, and it is doubtful whether the incidence of the disease was really affected. The numbers of cases and their severity probably vary from time to time. Steam cooking is used at both prisons but the disease occurred before this when rice was cooked in open "kwalis" (pans).

The prison diets (Appendix, page 134) have on the whole been carefully planned so as to provide what appeared to be a sufficiency of the known food requirements of human beings, including vitamins. Part of the vegetables provided are of the green leafy variety and probably form the main source of vitamins A and C. The anti-neuritic factor has been well provided for in view of the previous prevalence of beriberi. Diet C may be criticised as having no protein of a high biological value and this diet, and probably diet B, is low in the pellagra preventive factor or vitamin B2. The prisoners do not complain of their diet nor is there any loss of weight in the patients or in other prisoners in general. Prisoners on any of the three diets given may get the disease.

At the survey of Johore Prison made on 29th August, 1933, there were

26 prisoners working as cooks and only 2 of these had the disease; there were 15 prisoners working as dhobies (launderers), the washing being done close to the kitchens, and only 2 of these had the disease. At Singapore Prison also the cooks were practically all free from the disease. This comparative immunity of those working in or near the kitchens when compared with the prevalence in other parts of the prisons suggested that these men were appropriating small amounts of food extra for themselves and so unknowingly just escaping the disease.

Between 1931 and 1933 it had been noticed in the Johore Prison that *marmite* appeared to benefit some cases. This was accordingly investigated and the results were dramatic. During the second half of 1933, there were 60 cases of the epithelial disease treated at Johore by half an ounce (15 grams) of *marmite* by weight daily, given in water; in two weeks in all cases there was definite improvement, and in a month the disease of both mouth and scrotum, had practically disappeared in all 60; some of the cases (about 10) had obvious ringworm in addition and an ointment (a modification of Whitfield's ointment)* had to be applied twice daily to the patches before the lesions disappeared. Within a month of stopping the *marmite*, 20 of the 60 cases relapsed.

Further experiments were made at Singapore and Johore. Ten cases of eczema of the scrotum with stomatitis were given first *cod liver oil*, two ounces daily for four weeks, and did not improve. They were then given the juice of two oranges (equivalent to one ounce of *orange juice*) daily for three weeks, and did not improve, some of them getting so much worse that it could not be given longer. The same 10 were then given $\frac{1}{2}$ ounce *marmite* daily and began to improve rapidly, most becoming nearly well in two weeks; 9 of the 10 were completely cured after 26 days of *marmite*, the tenth still having slight redness of the scrotum only.

Autoclaved marmite.—Ten cases of eczema of the scrotum with stomatitis were treated with *marmite* autoclaved in an electric autoclave at 120° C. for five hours; an amount equivalent to $\frac{1}{2}$ ounce fresh *marmite* was given daily, 3 of the 10 were given the modified Whitfield's ringworm ointment to apply to the scrotum in addition and in one month all were cured except one who still had slight roughness and itchiness of the scrotum.

Brewers' yeast† was tried in the following experiments:—

A.—Ten cases were given 2 ounces of fresh yeast daily for 28 days, another 10 were given 2 ounces daily of autoclaved yeast (at 120° C. for 5 hours under 20 lbs. steam pressure) for the same time, and a further 10 cases were used as controls without treatment.

B.—The dose of yeast was increased to 4 ounces daily, and the results were much better.

*The ointment used was Ac. Benzoic. grains xxx, Ac. Salicyl. grains xxx, Paraff. moll. ad. oz. i.

†We are indebted to the Malayan Breweries, Ltd., for a constant supply of fresh yeast. The yeast, as used, was mixed with a considerable amount of fluid and samples varied somewhat; 4 ounces of this yeast probably represents about 18 grammes dried yeast (based on Kjeldahl estimations of nitrogen kindly performed by Professor ROSEDALE).

Yeast Experiment A.

			Cured.	Improved.	No Improve- ment.	Discharged before end of Experiment.
Fresh yeast, 2 oz. daily	2	3	3	2
Autoclaved yeast, 2 oz. daily	4	6	—	—
Controls, untreated	—	4	5	1

Yeast Experiment B.

			Cured.	Improved.	No Improve- ment.	Discharged before end of Experiment.
Fresh yeast, 4 oz. daily	7	1	2	—
Autoclaved yeast, 4 oz. daily	7	2	—	1
Controls, untreated	—	2	8	—

Later experiments confirmed the value of the yeast and the following figures are taken from various experiments where the yeast was used as treatment in control of the action of other substances, as compared with control cases on no treatment.

Yeast Experiment C.

			Cured.	Improved.	No Improve- ment.	Total Cases.
Fresh yeast, 4 oz. daily	26	3	—	29
No treatment	2	4	24	30

(In these three experiments the condition of the scrotum was taken as the test lesion.)

Liver.—Ten cases were put on $\frac{1}{2}$ lb. of cooked liver daily, 9 were cured within 25 days, the tenth in 34 days. The immediate improvement with liver was greater than that with yeast or marmite.

Thus, as regards vitamins, whereas foods containing only vitamins A, C or D, did not help the disease, those containing only vitamin B were found of very definite value. The fact that autoclaved yeast and marmite were curative suggests that it is vitamin B₂ which is the missing factor; it has been generally accepted that autoclaving destroys vitamin B₁ but not vitamin B₂.

Maintenance doses of marmite.—As has been mentioned, after marmite is stopped the disease tends to recur in many cases in about a month. Accordingly five cases of the disease after being cured by marmite, were given a maintenance dose of $\frac{1}{2}$ ounce daily for 15 weeks and remained well. The dose was then reduced to one drachm daily and three of the five had a recurrence of the disease within 6 weeks, but were soon cured by putting the dose up again to $\frac{1}{2}$ ounce daily. It thus seems that the maintenance dose is not very far short of the curative dose.

Local treatment.

It has already been mentioned earlier in this paper that a fungus infection may occur in these cases and it seems quite possible that some low-grade infection or a true epidermophyton infection plays a part in the disease. If this is so the infection probably acts as an irritant, or on the other hand an eczematous scrotum

may be peculiarly susceptible to the epidermophyton. The ringworm ointment that has already been mentioned (modified Whitfield's ointment) or a similar ointment of half this strength is often successful in curing patients if applied regularly for one or two weeks. Plain vaseline has been tried also to compare it with the antiseptic ointment but it was found of little value. Groups of cases examined vary considerably in respect to benefit received from local treatment but the severe typical case rarely responds. Professor ROSEDALE has kindly permitted us to say that in a recent experiment on the feeding of rats on vitamin B2 deficient diets he found a mangy condition of the fur develop which, however, could be cured by the local application of eight parts of rape oil with one part of black sulphur.

B.—STAGE OF NERVE LESIONS.

The incidence of this disease, its time of onset and its relationship to the epithelial disease have already been discussed. A certain number of pathological examinations have been made mainly with a view to comparing it with similar nervous diseases.

1. *Blood Wassermann reaction*.—Of 22 cases taken at random 15 were negative and 7 positive; of 100 other prisoners taken at random, 17 were positive and 3 were doubtful. Though the proportion of positive reactions is greater among nerve cases the figures do not suggest that syphilis is a cause.

2. *Cerebrospinal fluid*.—Of 8 cases taken at random at Singapore Prison all cerebrospinal fluids gave negative Wassermann and Khan reactions and negative colloidal reactions. The highest cell count was 8 per c.mm. The globulin test was recorded in 4 only and was negative.

3. *Gastric analysis*.—Of 17 fractional test meals taken at the two prisons 4 showed achlorhydria. Histamine stimulation was not used in any of the cases.

4. *Blood examination*.—No cases have been observed suffering from severe anaemia. Blood counts have frequently shown a mild degree of anaemia such as is common among the poorer members of the population. These cases have all been secondary in type and we have not observed any blood picture among these patients suggestive of pernicious anaemia.

EFFECTS OF TREATMENT.

Attempts at treatment of the subacute combined degeneration proved, as expected, somewhat disappointing, and this is in line with the experience of most workers in this disease, whether due to pernicious anaemia, pellagra, or any other cause. All the cases tended to improve on making them do what work they could; one of their chief difficulties in working was poor vision, and this was often definitely benefited by treatment. Four early cases of poor vision

who were given half pound of cooked liver daily for 6 months improved as follows :—

	Vision Test at Beginning.	Vision Test after 6 Months' Liver Treatment.
Case 1	R 1/60 L 1/60	R 6/ 9 L 6/ 9
Case 2	R 3/60 L 6/60	R 6/36 L 6/ 9
Case 3	R 6/24 L 6/60	R 6/ 9 L 6/ 6
Case 4	R 6/24 L 1/60	R 6/18 L 6/36

Iron, marmite, yeast, liver, and cod liver oil were all tried in treatment. Liver seems the most beneficial of these but we have not as yet done sufficient work to form conclusions on these forms of treatment.

DISCUSSION.

We have described a disease occurring in large numbers of the inmates of prisons and other institutions in Malaya and occurring sporadically amongst the general public. The disease is curable in the early stages by the use of yeast or marmite, fresh or autoclaved, and is, therefore, presumably due to a deficiency in the diet of the vitamin variously called B2, G or the PP factor. We find no support for the view that any of the symptoms are due to a lack of fat-soluble vitamin but to exclude altogether other dietetic deficiencies would perhaps be unwise, for a diet ill-balanced in one ingredient is quite possibly ill-balanced in others. The possibility of a toxic excess of cereals or of an unrecognized food deficiency cannot be easily excluded. The evidence we have put forward points particularly to the lack of vitamin B2. The relationship of the disease described (which seems very like GOLDBERGER's experimental pellagra) and classical pellagra is interesting, but a little obscure. As far as our knowledge goes the dietetic deficiencies in the two conditions are similar; yet typical pellagra does not occur in Malayan prisons, though sporadic cases are found in the general public. VISWALINGAM (1917, 1918, 1929) was the first to describe it in Malaya. Three cases of typical pellagra among several others seen recently by us, may be briefly described here; one was a hawker, one a fisherman and one a rickshaw puller. They were all Chinese and polished rice was the staple article of diet. The first two of these cases showed the typical distribution of pellagra pigmentation and dermatitis on the dorsum of the hands and feet, legs and arms, and back of neck. They also had diarrhoea with blood and mucus in the stools. The hawker had a stricture of the rectum and the fisherman on sigmoidoscopic examination showed ulcers resembling those of chronic

amoebic dysentery, though no amoebae or cysts could be found. Possibly the rectal ulceration was pellagrous.

The rickshaw puller had pigmentation and dermatitis on the hands and feet and on the back of the neck, but also had sealy dermatitis of the scrotum and stomatitis at the angles of the lips. In addition to these symptoms he had dilatation of the heart and absent knee jerks. This patient thus had the symptoms of pellagra *plus* those of the syndrome we are describing *plus* those of beriberi. Photographs of this case are reproduced (Plate II, Figs, 4 and 5) which show the condition of the hands and feet fairly well but which unfortunately do not reproduce the scrotal condition well. All these pellagra cases lost their symptoms rapidly with marmite treatment.

GOLDBERGER, WHEELER and other workers have found that dermatitis of the genital or anal region and stomatitis often occur in pellagra. HUTTER, MIDDLETON and STEENBOCK in a recent paper (1933) have brought forward evidence that the atrophic or sore tongue seen in such conditions as pernicious anaemia, sprue, pellagra, etc., is due to a deficiency of vitamin B either in the diet or owing to poor absorption in the alimentary tract. We have not found any cases of dermatitis of the hands or feet in our prison cases although some of the men work in the sun. Possibly pellagra may be uncommon in Malaya because the humid atmosphere cuts off much of the burning action of the sun. Whether the difference between pellagra and the disease we describe is one of external irritant or dietetic or toxic or climatic is at present but speculation. On the other hand we appreciate that there is much resemblance between the disease we have described and pellagra, but we feel that it would be incorrect to use the term pellagra for it and prefer to consider it avitaminosis B2.

SUMMARY.

1. A disease occurring in Malaya, particularly in institutions, is described. The main lesions in the early stage are eczema of the scrotum, eczema of the angles of the mouth and superficial glossitis. In the late stage the symptoms are those of combined degeneration of the cord and poor vision. A brief survey of the literature shows that symptoms of this type are widespread throughout the world.

2. The etiology is discussed and evidence given to show that the early stage is due to an avitaminosis B2 and that the late stage is probably due to a similar deficiency.

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APPENDIX.

SINGAPORE PRISON DIETS FOR CHINESE.

" A " DIET.

Rice	16	oz.
Bread	4	"
Vegetables	12	"
Fresh Meat	4	"
(Four times a week pork and three times a week beef.)							
Fish	3	oz.
(Four times a week fresh and three times a week salt fish.)							
Dhal* (Once a week only)	2	oz.
Cocoanut oil	1	"
Curry stuff	1	"
Salt	$\frac{1}{2}$	"

" B " DIET.

Rice	20	oz.
Bread	4	"
Vegetables	6	"
Fish	4	"
(Four days a week fresh and three days a week salt fish.)							
Dhal*	3	oz.
Cocoanut oil	$\frac{1}{2}$	"
Curry stuff	1	"
Salt	$\frac{1}{2}$	"

" C " DIET.

Rice	20	oz.
Bread	4	"
Vegetables	6	"
Beans (<i>soya</i>)	5	"
Cocoanut oil	$\frac{1}{2}$	"
Curry stuff	1	"
Salt	$\frac{3}{4}$	"

*The small green pea, *Phaseolus radiatus*, is used for Chinese.

A NOTE ON PERIODIC BANCROFTIAN FILARIASIS.

BY

CLAYTON LANE, M.D. (LOND.), LT.-COL. I.M.S. (RET.).

This note is an attempt at measuring the value of papers on infection with periodic *Wuchereria bancrofti* which have been made public in these TRANSACTIONS during the last three years.* In doing so comparisons have had to be made with other papers in other journals.

THE OPTIMUM HABITAT OF THE ADULT WORMS.

O'CONNOR and HULSE (1932) gave an account of a boy in whose night blood *Microfilaria bancrofti* was present and who had a right inguinal swelling. After this had been cut out by KLEIN between 1.43 and 2.28 p.m. it was seen to be

*It is, in addition, an attempt to put the argument into "Basic English," as a step towards the international standardisation necessary for further simplification so far as one with little experience has been able to do so.

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(Four times a week fresh and three times a week salt fish.)

Dhal* (Once a week only)	2	oz.
Cocoanut oil	1	„
Curry stuff	1	„
Salt	$\frac{1}{2}$	„

“ B ” DIET.

Rice	20	oz.
Bread	4	„
Vegetables	6	„
Fish	4	„

(Four days a week fresh and three days a week salt fish.)

Dhal*	3	oz.
Cocoanut oil	$\frac{1}{2}$	„
Curry stuff	1	„
Salt	$\frac{1}{2}$	„

“ C ” DIET.

Rice	20	oz.
Bread	4	„
Vegetables	6	„
Beans (<i>soya</i>)	5	„
Cocoanut oil	$\frac{1}{2}$	„
Curry stuff	1	„
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made up of five lymph glands, in or by the side of which 21 adult worms of this species were present—11 dead, and 10 healthy and clearly living at the time of operation. Again just this mixture of dead and living worms was present when O'CONNOR, GOLDEN and AUCHINCLOSS (1930) cut out "focal spots," that is to say those places which may give pain during an attack of lymphangitis or elephantoid fever in filarial persons, specially in the thighs or legs; and in their hands X-rays made it clear that shadows, such as are made by calcified worms, came from bodies in Hunter's canal, and moreover that fifteen like shadows or groups of shadows were present in one elephantoid leg. That their view of the cause of these shadows was right became clear when some of the focal spots which had made them were cut out and calcified worms were in fact seen within them. Again HAMILTON FAIRLEY (Discussion, in O'CONNOR, 1932) gave an account of the discovery of worms in a focal spot taken from the right arm. As to the lymph glands O'CONNOR gave detail to the matter by his statement that the habitats of 75 per cent. of these filariae were in the lymph vessels round the glands, or in the capsule and the cortical sinuses of the glands. While it is true that the most common place in which the worms are seen is within the scrotum, in order to be sure that they are frequently present in other places there is no need to go beyond these TRANSACTIONS. They have, however, seen two opposite views given on this point. GRACE (1934) said, "Our conception of these 'focal spots' is that they are due to tissue reaction to the presence of bacteria, and that the worm is there because there are so many in the tissues, and that it is dead because it has been killed by the bacterial toxin." Evidence was not given by him then, and has not been given by anyone else, that worms are in fact present in such large numbers. ROMITI's quite opposite opinion (1935) is this. "The site where the worms are always found is the same *viz.* in the distal portion of the lymphatic plexa [sic] of the cord, in proximity to the epididymis. . . . Immature living worms were found in a few instances in the lymph glands draining the genital organs [which are the lumbar glands] and in every case the presence of the worms was the cause of important clinical manifestations. . . . The author has not been able to find adult worms in any other situation, nor to observe in any other district of the lymphatic system, the lesions which are characteristic of invasion by the adult worm." Moreover, having seen worms making their way out of tissues which had been cut out of the living body and put into normal saline, ROMITI's view is that they did so because of fall in temperature and clotting of lymph, and that they will make their way equally readily within the body from superficial to deep lymph vessels after death if these are open. The sense of all this seems to be that if adult worms are found outside the sex organs they have got there after death. That is not so, as one example of many makes very clear. In a Demonstration before this Society of sections made by O'CONNOR there was put out one (LANE, 1934) of a left inguinal gland cut out at operation by KNOTT at 11.30 a.m. and in it was seen a female worm, whose uteri were full of outstretched microfilariae. Quite

certainly the worm was there during the man's life, and, as already said, the passing of lymph from the epididymis or cord is not into the inguinal glands. There is no ground at all for the statement that worms, present after death in places other than the cord or epididymis, had their habitat in those special parts of the sex system during life.

These theories take no account of known facts. The writer's own (LANE, discussion, in O'CONNOR, 1932) is that after an infective larva from the mosquito has made its way into the skin of man, it has before it the lymph and blood escalators. If it takes the lymph escalator it may come to a stop somewhere in the slow lymph current, and there grow to adult condition; either somewhere in the arm, thigh or leg as is seen from GOLDEN's X-ray work, or at the first lymph gland to which it comes. By the blood current infective larvae are at once taken off to the right heart, lungs, left heart, and so to all parts of the body; the capillary to which such a larva is taken is "suitable," whatever that very old condition means, it makes its way through the walls into the tissues, apparently into a lymph vessel, and becomes full grown there; if it is not, it is taken on through the veins, till by the blood current it comes to some place which is so, or till it dies. By this theory the behaviour of these larvae is that which would reasonably be looked for from our knowledge of that of others in like conditions; it makes clear how it is that in males most worms are present in the organs of sex.

A FORWARD MOTION OF MICROFILARIAE OR THEIR CARRIAGE BY BLOOD OR LYMPH?

The birth of microfilariae takes place on the lymph escalator, but it is in the blood that their periodicity is commonly seen. They can come to the blood current either by the use of the whole of the lymph escalator up to its opening into the vena cava, or by taking a short cut through the walls of both escalators, as for example in a lymph gland where they seem specially close to one another. But can the lymph current take microfilariae through a lymph gland and from the incoming to the outgoing vessel? I have made the suggestion (LANE, 1933) that it cannot, because O'CONNOR had never seen microfilariae in the outgoing lymph vessel, because DRINKER (1933, and in LANE, 1934) had given such beautiful evidence of the spider's web qualities of the glands in the trapping of bacteria, because larvae, often clearly dying, have been seen in them in great numbers, and because the glands in this infection finally become little but a network of wide lymph vessels, the main lymph current not going through their working parts at all. It has seemed to me that only through such "bypassing" of the true gland substance can microfilariae get into the blood, in other words, that a long "latent period" is necessary between the time when these became adult and that at which their young are first seen in the blood. The reason given by ROMER (*l.c.*) for the fact that children are free

from infection is different. "It is only when the sexual organs [of man] have reached maturity that *Filaria bancrofti* finds the *normal* habitat to complete its *normal* cycle." The reasons, for which I cannot give my agreement to the opinion that the sex organs are the necessary site of mature worms, have been put forward above; the facts are not against my own theory.

On the other hand DRINKER, AUGUSTINE and LEIGH (1935) say: "The microfilariae of *Dirofilaria immitis* pass through normal lymph nodes with great ease. . . . By analogy it is suggested that the microfilariae of *Wuchereria bancrofti* in the lymph stream will not be measurably impeded by lymph nodes in their journey to the blood stream." The meaning of the first part of this statement seems to be that *Mf. immitis* goes with little trouble through a gland in the lymph from the incoming to the outgoing lymph vessel. But from Table I of their paper it is clear that, when 16,141 of these larvae were put into the incoming lymph vessel of the popliteal gland during 85 minutes, only 125 got as far as the outgoing vessel, in other words there was a loss from the lymph of 99.2 per cent. of them. Again from their Table II it will be seen that of 88,252 microfilariae put into the incoming lymph vessel of another lymph gland during 114 minutes, 1,400 got as far as the thoracic duct, that is to say no account could be given of 98.4 per cent. of these tens of thousands. The statement, then, that *Mf. immitis* can "pass through normal lymph nodes with great ease," has not on its face the sense which the ordinary reader will give to it. Clearly the microfilariae were not let through the gland on the lymph escalator. Three different things may have taken place; they were kept back in the gland undamaged, or they underwent destruction there, or in some way they got off the escalator. These writers make note of the small numbers of microfilariae which can be seen in sections of these glands; Professor DRINKER has most kindly sent me some of them, and I am certain microfilariae are not there in the great quantities in which they should be had they simply been kept back in the glands. There is no evidence of their destruction. Seemingly, while in the gland, they have made their way from the lymph to the blood escalator. That the last is so, seems probable from these writers' statement that *Mf. immitis* is present in numbers in the lymph in different parts of the body. Since it can by its own act get from its birth-place in the blood to the lymph, it should be able in the same way to get from lymph to blood, specially in the lymph glands where the escalators do not always seem to be quite separate. At all events, whatever the reason, it is in harmony with the facts to say that only with great difficulty can *Mf. immitis* get from the incoming to the outgoing lymph vessel of a lymph gland.

Next these writers make the suggestion that by analogy *Mf. bancrofti* "will not measurably be impeded by lymph nodes in their journey to the blood stream." The reason for this analogy is partly the writers' opinion of the behaviour in like conditions of *Mf. immitis*, which as just seen, I take to be a wrong one; partly on the fact that when mapping out its journey on a blood slide the rate

of forward moving of *Mf. immitis* was 0·14 to 0·19 mm. a minute, whereas that of *Mf. loa* was 0·37 mm. a minute.* If *Mf. bancrofti* can by its own act normally make its way through lymph glands without the loss of its sheath, how is it that other sheathed larvae such as those of hookworms cannot do so when the tissue in question is the skin? For it is a commonplace that the sheaths of these may be seen in numbers on the skin into which larvae have made their way and another that *Mf. bancrofti* in fresh blood is so nearly always sheathed that to come across it otherwise, as in some of KNOTT's experiments, is something for special note. It does not seem possible that these microfilariae should so regularly get into the blood, complete with sheaths, if their normal way of doing so were by force through even such a loose structure as that of the sinuses of lymph glands, for even there it is probable that their known movements will send the loose ends of their sheaths whipping round the fibres in the sinuses, and that they themselves will get away only after they have broken through these. An observation by DYCE SHARP (1927) should be kept in mind here. The normal habitat of *Mf. volvulus* is lymph, and his words are "The embryos are commonly found in the lymph glands of the groin and elsewhere, sometimes in enormous numbers." Arguments by analogy, even from one sort of unsheathed larva to another, may, then, lead to quite wrong conclusions, those from unsheathed to sheathed larvae are even less safe.

REACTIONS OF MICROFILARIAE AND LYMPH GLANDS TO ONE ANOTHER.

On the question of the analogy between *Mf. immitis* and *Mf. bancrofti* it has been noted that these writers saw no evidence of damage to *Mf. immitis* in lymph glands, and no reaction in these structures to the larvae. But in the case of *Mf. bancrofti* it was beyond question that their destruction was taking place and that its effects could be seen in sections made by O'CONNOR and put out at this Society's Meetings (LANE, 1933, 1934), for there were present in them calcification or feeble nuclear staining on the one hand, and on the other the sinuses were full of cells, and there was a fibrosis which was clearly a later effect. The point of this discussion is that analogies between the behaviour of *Mf. bancrofti* and other microfilariae in experimental or natural infection, and the reaction of the tissues to these, cannot be truly or safely made.

PERIODICITY.

In KNOTT's summary (1935) the chief points are taken to be these. It is hard for microfilariae to go through capillaries from arteries to veins, moreover,

*These numbers have no relation to the idea that *Mf. bancrofti* can keep its position in the heart in the very much quicker current in existence during contraction. It has not even a rheotaxis.

being less strong in day blood than in night blood they are kept back in capillaries by day but get through them by night because of their greater power then, and this he says is the mechanism of their night periodicity. As to their different activities by night and day, this observation was, it seems, first made by MYERS (1881) whose account of the daytime microfilaria was, "The contrast with the vigorous, rapidly-moving organism seen at night was very marked," for by day all were weak and became outstretched. It is an observation on which, however, there does not seem to be agreement, and I have no knowledge of any recorded observation that these animals, if kept living outside the body for more than twenty-four hours, are more active by night than by day. But KNOTT's own work makes it clear that this theory of the cause of microfilarial periodicity cannot be right. If the amount of man's blood is taken as being 3,000 c.c., then KNOTT put into the blood vessels of Case S.G. enough microfilariae to make up 283 in every c.c. of his blood, but none were ever seen again even after looking through 25 c.c. of blood, 5 c.c. of this being taken from him five times during 48 hours. Now this injection of microfilariae was given to S.G. at 10.40 p.m. at a time that is when the amount of motion of the larvae should have been near its greatest; while the blood, and they in it, had been outside the body of man for ten minutes only. If their periodicity were dependent on their own acts they should have been seen in the blood at once. They were not seen at all.

As to the observation that microfilariae are kept back in the capillaries, KNOTT's new work is an agreement with that of WARRINGTON YORKE and BLACKLOCK (1917), though the opinion of these as to the mechanism at the back of things is quite different. "Microfilariae were to some extent held up in their passage through the cutaneous vessels. . . . Obstruction to the passage of the larvae through the cutaneous vessels undoubtedly aids in the piling up of larvae in these vessels, but this factor operates at all hours of the day and night and is in no way responsible for the nocturnal periodicity."

KNOTT gives details of a technique for the treatment of a large amount of blood in such a way that any larva in it comes into view. Use was made of this technique after the injection of microfilariae into the blood, but in no case does his language make clear that it was in use before the injection. The suggestion from the wording of the paper is that it was not. If so his method before microfilariae were put into man was a rough one and after that event a delicate one, so that no right comparison between the numbers of larvae seen in the two groups of observations can be made.

A word must be said about two more of KNOTT's experiments. Case C.A. had an infection with 294 microfilariae in each c.c. of his 3,000 c.c. of blood; the addition of more at the rate of 700 for each c.c. of his blood gave numbers of 276, 256, 232, 280, and 248 per c.c. in five samples taken during the next hour; all the numbers were less than those seen before this nearly three-fold addition. Case E.D., of 18th March, 1933, came from an island where filariasis was endemic and may well be an example of the effect of the use of different

techniques for numbering microfilariae before and after the injection of infected blood. Moreover, there is little knowledge as to whether or not injection of blood, free from microfilariae, into a man in whose body they are present in small numbers, may in some way make for an increase in numbers in the blood, for it is a fact of great weight that we have so far no knowledge of the stimulus which is the cause of periodicity in filariasis, though in my belief we now have some idea of the mechanism itself.

As to this, KNOTT's opinion is that in O'CONNOR's serial sections made on a mass scale there is proof that in periodic filariasis, all mother worms in the body give birth to microfilariae about the same hour, but that as pointed out by MANSON-BAHR (1929) "the crucial point in Lane's theory is the actual survival time of the microfilariae." In my view the real question is whether all female worms in this infection do or do not give birth to their young at the same hour every day. Quite certainly this was MANSON's too, for he said, "Periodicity may be explained by one of two suppositions: (a) the parent worm empties her uterus of mature embryos once every 24 hours, parturition going on from late in the afternoon till midnight*, the young filariae live for but a few hours in the blood and are then disintegrated, as Dr. MYERS (of Formosa) has suggested; (b) parturition is a more or less continuous process—the young being nearly constantly carried along the thoracic duct into the blood. . . . The facts I have stated and other evidence lead me to believe that the second hypothesis is the correct one, *viz.*, that reproduction is continuous and that the embryos are fixed in some organ or tissue during the day" (quoted by MANSON-BAHR, 1935). In MANSON's belief, then, there were only these two opposite possibilities, and the second was the true one. In KNOTT's the first is right; what room is there, then, for the second? If the birth of microfilariae takes place at the same hour every day there is complete enough reason for their coming into the blood in a wave. As to the ending of the wave, KNOTT's work is a clear addition to our knowledge of its mechanism, but gives none as to the place where destruction of microfilariae takes place, nor how it comes about. It need not be in the capillaries generally. For example, TOPLEY (1933), gives an account of how bacteria present in the blood are at first kept back in the lung capillaries, where they are taken up by polymorphonuclear white cells. These, together with the bacteria within them are taken off by the blood to reticulo-endothelial "depots" in spleen, liver and other places, where they together with the bacteria inside them become in turn food for sessile histiocytes. The question as to the place of destruction of microfilariae cannot but be hard to answer. To make clear that where there is now nothing there had once been something is necessarily so, but the attempt has to be made. It is as well not to be too sure that the mechanisms which the body certainly has in use against bacteria are those of greatest value against worms. After all the effects of

*The hour is about mid-day as was made clear by O'CONNOR's sections of worms whose death took place at 11.30 a.m. and about 2 p.m. (LANE, 1934).

lysozyme are real, and ficin can make parts of living ascaris into a liquid and nothing more.

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MENINGITIS DUE TO MUCOID-ENCAPSULATED BACILLI.
REPORT OF TWO CASES.

BY

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INTRODUCTION.

This paper deals with the laboratory findings of two fatal cases of meningitis recently encountered in Hongkong, the causal organisms being identified respectively as *Bacterium aerogenes* and *Bact. friedländeri*.

Whether these organisms should be assigned to the same group appears to be a controversial question. As TOPLEY and WILSON (1931) point out, although the exact nature of the relationship existing between the two organisms is unknown there is a preponderance of opinion that they are related. FORD (1927) classes them together in the mucoid-encapsulated group. There appears to be general unanimity of opinion concerning the criteria for the differential identification of *Bact. aerogenes*, namely the production of acid and gas in lactose and most other carbohydrates commonly employed, inability to ferment dulcitol,

*We wish to record our indebtedness to Dr. D. K. SAMY and to Professor W. I. GERRARD, O.B.E., for the clinical data of Case 1 and Case 2, respectively, and to Dr. G. H. THOMAS for the postmortem findings in Case 1.

positive Voges-Proskauer reaction, negative methyl red reaction, inability to form indol but ability to grow in citrate broth. The behaviour of different strains of *Bact. friedländeri* to the usual biochemical tests varies so widely, however, that no particular set of criteria serves for the positive identification of this organism. Its reaction to each of the first four tests mentioned above is conflictingly described by various authors, HEWLETT (1926), BERGY (1926), STITT (1927), FORD (1929), MACKIE and MCCARTNEY (1934), TOPLEY and WILSON (1931), and SHAW (1931). There is nevertheless general agreement that the organism is a Gram-negative encapsulated non-motile bacillus, characterized by a mucoid growth on agar, unable to liquefy gelatine, but capable of fermenting most of the carbohydrates commonly employed with production of acid and, in the case of most strains, gas.

References could be found to only four previous cases of meningitis due to *Bact. aerogenes*. It is not improbable, however, that owing to inadequate bacteriological criteria this organism has occasionally escaped proper identification and been classed as *Bact. friedländeri*. In the earlier literature SCHIEB (quoted by ABEL and HOLLWACHS, 1913) and BEITZKE (quoted by SHAW, 1931) record cases apparently due to *Bact. aerogenes*. More recently DEANE and SHERA (1928) in England, and SHAW (1931) in U.S.A. described fatal cases due to this organism.

Of reported cases ascribed to *Bact. friedländeri*, ROTHSCILD (1931) gives a comprehensive survey, and himself reports a non-fatal case. The bacteriological data set forth in this author's paper would, however, hardly warrant the exclusion of *Bact. aerogenes* as the causal organism. Unfortunately, most of the earlier papers reviewed by ROTHSCILD appeared in Continental journals not available here. It would seem that the total number of previous cases falls short of twenty. RUSSEL BRAIN and VALENTINE (1928) described a fatal case of meningitis due to an organism they identified as *B. mucosus capsulatus*. It was a non-motile encapsulated Gram-negative bacillus forming acid but not gas in dextrose, mannite and lactose, failing to ferment inulin and dulcitate, acidifying but not clotting milk, and virulent to mice. It will be seen that its reported characteristics closely correspond to those of the organism isolated by us from Case 2. KLIEWE (1928) isolated from a fatal case of meningitis in a two months old infant, a mucoid encapsulated bacillus. This organism, however, liquefied gelatine. KLIEWE named it *Bact. mucosum mutabile*.

CASE 1.

A Chinese female aged 29, 8 months' pregnant was admitted to a maternity hospital complaining of having felt no foetal movements for 4 days. Five days later she was delivered of a macerated foetus. During and after labour she developed pyrexia and after a further 5 days meningeal symptoms appeared. She was thereupon transferred to the general hospital where she died the same evening. An autopsy was performed by Dr. G. H. THOMAS, who kindly informed us that the brain, especially the vertex was covered with thick viscid greenish exudate, and that no gross pathological lesions were encountered in the other organs.

Bacteriology.

A sample of the cerebrospinal fluid taken the day before death was immediately sent to this laboratory where it was examined within half an hour of its receipt. It was found to be turbid. Examination of a film showed many polymorphonuclear leucocytes and numerous extra-cellular Gram-negative cocco-bacilli. The fluid was cultured aerobically and anaerobically on various media. In all cases a pure culture was obtained.

Three days before the patient's death a sample of the lochial discharge was also examined in this laboratory. Gram-negative bacilli and staphylococci were found in direct smears and on culture. The bacilli were subsequently found by all the criteria employed to be identical with the organism isolated from the cerebrospinal fluid.

Morphology.—The organisms were Gram-negative. In an 18 hour broth culture, stained with dilute carbol-fuchsin they appeared as cocco-bacilli. The coccoid forms appeared in pairs; the bacilli singly, in pairs and occasionally in short chains. The bacilliform organisms measured $1.5-3.5\mu \times 0.4-0.6\mu$. Capsules were readily demonstrated by various methods. The technique described by HOWIE and KIRKPATRICK (1934) was found to be very convenient and successful. Capsules were present in cultures as well as in animal fluids. The organisms were invariably non-motile. No spores were demonstrated.

Cultural Characters.—On agar plates after 24 hours aerobic incubation at 37°C ., the colonies were round, smooth, yellowish-white, convex with opaque centres and translucent entire edges, 2–4 mm. in diameter. In consistence they were mucoid and emulsified readily. After 7 days incubation the colonies became papillate. On agar slopes the growth was abundant, raised and mucoid. Anaerobic cultivation resulted in a poor growth. On McConkey plates, the colonies were pink, glistening, mucoid, low convex with an entire edge averaging 3 mm. in diameter. On rabbit-blood slopes, 24 hours at 37°C ., the growth was abundant, greyish-white in colour. On Loeffler's serum, 24 hours at 37°C ., the growth was white and mucoid, no liquefaction of the medium occurring. In nutrient broth, after 24 hours at 37°C ., the growth was thick, of uniform turbidity. A gelatine stab culture after 7 days at 22°C . showed growth along the line of the stab, with a somewhat heaped-up "nail-head" appearance at the surface. No liquefaction of the gelatine occurred, but gas bubbles formed. Potato slope culture resulted in a pale yellow mucoid growth with abundant gas formation in the water of condensation.

Biochemical Reactions.—These are set forth in the accompanying table.

Pathogenicity.—Mice: 0.01 c.c. of an 18-hour broth culture was fatal within 72 hours whether the inoculum was injected intraperitoneally, intravenously or subcutaneously. 0.001 c.c. was non-fatal. Guinea-pigs: intraperitoneally, 0.01 c.c. was fatal to one of two animals injected. Subcutaneously, 0.1 c.c. was non-fatal. Rabbits were resistant to 0.1 c.c. inoculated by any

route. In animals dying in these experiments the organisms were in all cases isolated from the blood stream. The most constant pathological findings were enlargement and congestion of the spleen and congestion of the adrenals. In animals dying following intraperitoneal inoculations, the exudate contained macrophages and a few lymphocytes, polymorphonuclear leucocytes were not seen.

TABLE.
BIOCHEMICAL REACTIONS OF ORGANISMS.

Test.	Case 1.	Case 2.	Test.	Case 1.	Case 2.
Glucose	A G	A	Sorbitol	A G	A
Galactose	A G	A			(in 3 days)
Lactose	A G	0	Salicin	A G	A
		(15 days)			(in 10 days)
Maltose	A G	A	Inosite	A G	A
Saccharose	A G	0	Litmus Milk	A, clot	Alk, late
		(15 days)	Indol Formation	0	+
Arabinose	A G	A	Voges Proskauer Reaction	+	0
Xylose	A G	A	Methyl Red Reaction	0	+
Inulin	A G	A	Citrate Utilization Test	+	0
Dextrin	A G	A	Nitrate Reduction	+	+
Mannitol	A G	A	Haemolysis (Rabbit's cells)	0	0
Dulcitol	0	A			
	(15 days)				

Serology.—An agglutinating serum was prepared against the organism isolated from the cerebrospinal fluid. The titre was 1/250 after five injections into a rabbit. The organism isolated from the lochia was agglutinated to titre by this serum.

COMMENT.

The morphology, cultural characters and biochemical behaviour of this organism in our opinion warrants its being identified as *Bact. aerogenes* (*Aerobacter aerogenes* Beijerinck).

The history of this case, and the bacteriological findings in the lochia, suggest that the infection of the meninges may have been secondary to general or to uterine infection.

CASE 2.

This was a Chinese male infant, aged 1 month, admitted to hospital 10 days previously with fever and a purulent discharge from both eyes. He developed squint, opisthotonos and Kernig's sign. A sample of the cerebrospinal fluid was submitted to this laboratory. It was found to be turbid and contained numerous mononuclear cells and Gram-negative

bacilli. The latter were very numerous occurring extra-cellular and intra-cellular, many of the cells being packed with the bacilli. The cells all appeared to be macrophages in various stages of development.

The patient died the day following the examination of the cerebrospinal fluid. Autopsy was performed by one of us.

Autopsy Findings.

The body was that of a well nourished infant. *Rigor mortis* was present. No signs of disease were noticed in ears, nose or mouth. No free fluid was found within the peritoneal cavity. The alimentary tract was empty and distended with gas. Liver, kidneys and adrenals appeared to be healthy. The spleen was congested but not markedly enlarged. Both lungs were consolidated at the bases. The heart, pericardium, myocardium and endocardium presented no morbid appearances. Cranium: the pia mater was covered with a thick greenish-yellow exudate all over the brain including the base. The cerebral vessels were congested. The spinal cord was covered with the same type of exudate as the brain. No evidence was seen of disease of the internal or middle ear.

Morbid Histology.

The pia mater was extensively inflamed, the vessels were engorged and the whole infiltrated with macrophages or histiocytes. The monocytic nature of the cellular response was a striking feature and is depicted in the Plate. Sections suitably stained (Gram, carbol-thionin and iron-haematoxylin) show numerous bacilli amidst the inflamed tissue. The brain tissue appeared to be affected to varying extent. Many of the cerebral vessels were congested. Sections of the bases of the lungs revealed extensive consolidation, the alveoli and bronchioles being filled with cellular exudate consisting of polymorphonuclear leucocytes and histiocytes. Suitably stained sections showed numerous Gram-positive diplococci and Gram-negative cocco-bacilli in the exudate. The histological appearances of the liver were those of venous congestion and fatty degeneration. In the spleen there was pronounced vascular congestion and endothelial hyperplasia. Examination of the kidneys revealed congested and swollen glomeruli, in many instances the capsular spaces being obliterated. The tubular epithelium was degenerated and the vessels congested. The adrenals showed vascular congestion of the medulla.

Bacteriology.

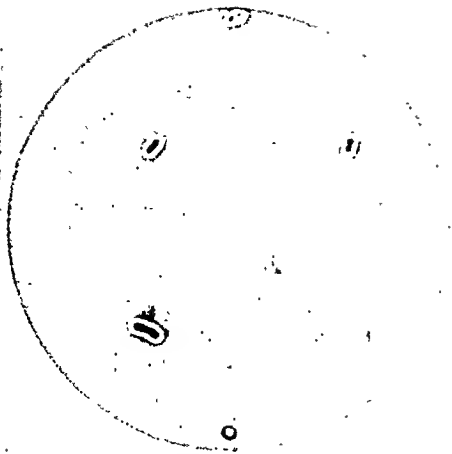
From the cerebrospinal fluid, *ante-mortem* as well as *post-mortem*, pure cultures were secured of the organism now to be described.

Morphology.—In an 18-hour broth culture, stained with dilute carbol fuchsin the organisms appeared as bacilli measuring $2-3.5\mu \times 0.4-0.5\mu$. Diploid forms were frequent. Capsules could be demonstrated by various

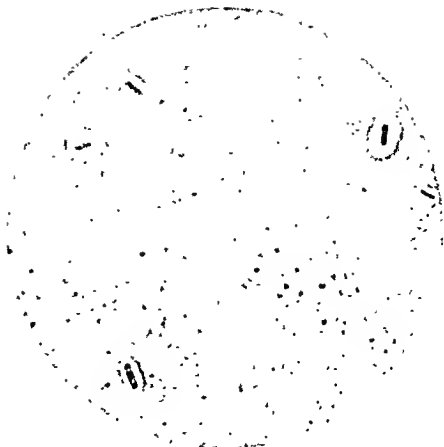
DESCRIPTION OF PLATE.

- FIG. Case 1. *Bact. aerogenes*, showing capsules.
(Howie and Kirkpatrick's stain) $\times 1100$.
- FIG. 2.—Case 2. *Bact. friedländeri*, showing capsules.
(Howie and Kirkpatrick's stain) $\times 1100$.
- FIG. 3.—Case 2. Monocytes in cerebrospinal fluid.
(Fixed in Schaudinn's fluid, stained H. and E.) $\times 1100$.
- FIG. 4.—Case 2. Inflammatory exudate over cerebellum. (H. and E.) $\times 130$.
- Fig. 5.—Case 2. High-power view of inflamed pia mater. (H. and E.) $\times 550$.
- Fig. 6.—Case 2 Showing nature of inflammatory cells in pia mater. (H. and E.) $\times 1100$.

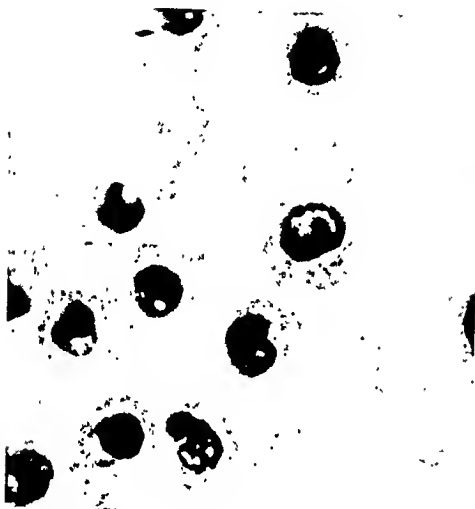
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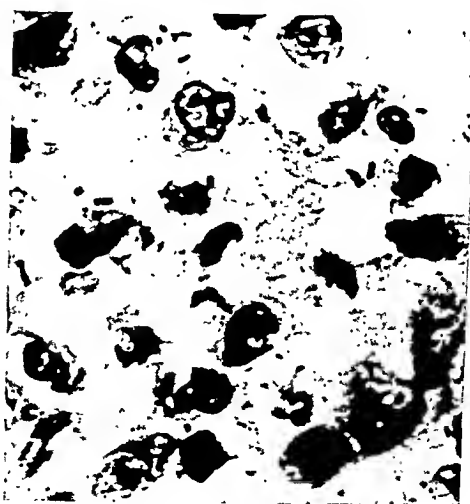
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methods, but they were on the whole less conspicuous in cultures than those seen in Case 1. The organisms were Gram-negative and were invariably non-motile. Spores were not demonstrated.

Cultural Characters.—On agar plates after 24 hours aerobic incubation at 37° C. the colonies appeared as dome-shaped discs with round entire edges, 1–3 mm. in diameter, greyish-white in colour, of soft creamy consistence they emulsified readily. The colonies were not so raised nor so mucoid as those of Case 1. On agar slopes the growth was abundant and mucoid, but under the same conditions, the growth was never as abundant as that of the organism in Case 1. On McConkey plates after 24 hours, the colonies appeared as small white domes 1–2 mm. in diameter. In nutrient broth a uniform turbidity was produced. Grown on potato slopes the culture was yellow, slimy without gas formation. Gelatine stab culture cultivated at 22° C. appeared as a white line of growth with a flattened “nail-head” at the surface, neither gas formation nor liquefaction occurred.

Biochemical Reactions.—These are set forth in the table on page 146.

Pathogenicity.—Mice injected intraperitoneally with 0.001 c.c. of an 18-hour broth culture succumbed within 24 hours. Injected subcutaneously or intravenously, 0.01 c.c. was necessary to cause death within 72 hours. In guineapigs, 0.01 c.c. was the minimal lethal dose intraperitoneally. Rabbits were resistant to 0.1 c.c. of an 18-hour broth culture inoculated by any route. These experiments were performed at the same time with the same culture. In mice and guineapigs dying following these inoculations, the organisms were in all cases recovered from the heart blood. The most constant pathological findings were enlargement and congestion of the spleen, and adrenals. Examination of sections of the spleen and in the case of animals inoculated intraperitoneally, of the exudate, showed that the cellular response was predominantly monocytic.

COMMENT.

It is considered that the characters recorded above justify this organism being classed as *Bacterium friedländeri*. Reference has already been made to the extreme variability of the biochemical reactions of different strains of this organism, which as TOPLEY and WILSON (1931) point out will ultimately probably require subdivision into several sub-types. SMALL and JULIANELLE (1923) attempted such a division on the basis of biochemical reactions and described four divisions. It would appear that their Division 3, comprising strains fermenting dextrose but not lactose or saccharose with or without formation of gas, corresponds to the organism we have described.

In this case there was a basal-pneumonia in which Gram-negative bacilli were seen microscopically. It is a matter of surmise whether this condition was primary or secondary to the meningeal infection. The clinical history would suggest the latter.

SUMMARY.

The bacteriological findings in two fatal cases of meningitis have been described. In the one case, a parturient Chinese female, the causal organism was identified as *Bact. aerogenes*, in the other, a Chinese male infant aged one month, *Bact. friedländeri*. The histopathology of the latter case has been described: the most striking feature was the histiocytic nature of the cellular response.

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CASE OF STREPTOTHIRICAL ULCERATION OF THE COLON WITH PORTAL AND SYSTEMIC PYAEMIA.

BY

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AND

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Streptothricosis is not a common disease. The number of instances in which the cultural reactions of the streptothrix have been adequately studied is not numerous. Furthermore, the classification of this group of fungi is by no means finally settled, and for this reason we felt the following case was worthy of detailed record.

I.—CLINICAL ASPECTS.

H. S. M., a missionary, aged 60 years, had spent 30 years in China. From 1904 to 1907 he had resided in the Anhwei Provinces (Central China), and the rest of the time in Shansi in Northern China.

Past History.—In August, 1916, he developed dysentery and was admitted in January, 1917, to hospital where he received so much emetine treatment that the heart was affected. Discharged two months later.

Arrived home from China 8/8/34 and was found to be medically fit except for some dental sepsis: extensive extractions were performed.

Onset.—Patient was quite fit until 23/10/34, when he developed pain in the right upper quadrant of the abdomen associated with shivering and a rise in temperature. The pain was not severe and recurred at intervals for about 3 days; there was considerable fever, but this gradually subsided, the temperature reaching normal on the 5th day. Four days later there was a return of epigastric discomfort and fever; since then there was a daily rise of temperature and a sense of fullness after eating. The stools appeared normal to the naked eye and the bowels tended to be constipated.

Subsequent Progress.—From 13/11/34 to 5/12/34 the patient was in the Hospital for the China Inland Mission under the care of Dr. J. W. JACKSON. There was at first a remittent and later an intermittent swinging temperature varying from 96.4 to 103° F. with a pulse rate of 80 to 128 per minute and respirations of 20 to 28 per minute. The tongue was moist and coated, there was no anaemia or jaundice and no demonstrable enlargement or tenderness of the spleen or liver. The Widal reaction was +1/125 for *B. typhosus*, but this was not regarded as significant; there was a leucocytosis, 85 per cent. of the cells being polymorphonuclear neutrophil leucocytes.

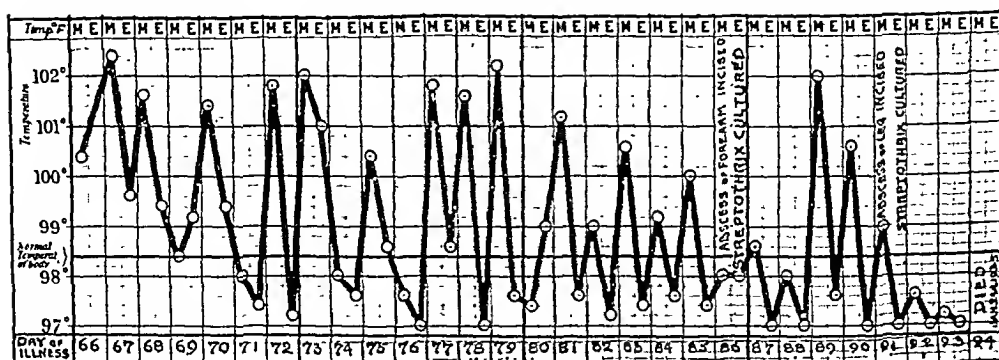
From 6/12/34 to 24/1/35 the patient was in the Hospital for Tropical Diseases where remittent and intermittent fever associated with chills and rigors persisted until a few days before death.

Physical Examination (6/12/34).—Examination showed an elderly man, well nourished and sweating profusely. The tongue was moist and furred. A few crepitations were present at the base of the lungs and the liver appeared somewhat enlarged downwards and definitely tender.

Laboratory Examination.—The faeces showed no amoebic cysts or ova, the urine was normal and the van den Bergh reaction was negative direct and positive indirect (2 units). Blood culture was negative. R.B.C's = 4,500,000 per c.mm., haemoglobin = 86 per cent. (Haldane); colour index = 1.0; leucocytes = 29,000 per c.mm., neutrophils = 85 per cent., lymphocytes = 12 per cent., monocytes = 3 per cent.

X-ray Examination.—The liver shadow was slightly enlarged, and there was slight elevation of the diaphragm though its movement was good. A dense opacity in the liver area suggestive of liver abscess was demonstrated.

In view of the history of amoebiasis, the tender liver, the X-ray findings and the marked leucocytosis, liver abscess was diagnosed. The liver was needled by Mr. McINDOE with



CASE OF STREPTOTHRICOSIS WITH ULCERATION OF THE COLON AND PORTAL AND SYSTEMIC PYAEMIA.

Temperature chart of last four weeks. Duration of illness 94 days.

negative results and subsequently a laparotomy was performed (8/12/34). The liver was congested and peculiarly mottled in appearance and felt hard and fibrosed. Needling failed to reveal pus and the contents of the aspirating syringe were sterile.

Biopsy.—A small piece of liver was excised for microscopic examination, and section showed the presence of periportal biliary cirrhosis suggestive of chronic fluke infestation (*Clonorchis sinensis*); there was an associated acute cholangitis with extensive polymorphonuclear infiltration of the bile ducts. Cultures, however, failed to reveal any pyogenic organism.

Post-Operative Progress.—Apart from some troublesome post-operative distension of the abdomen the illness was unaffected by surgical intervention. Rigors persisted and despite the negative blood cultures antistreptococcal serum was administered. Petechial haemorrhages were noted on the right arm.

16/1/35.—A localised swelling on the upper part of the left forearm was incised and later pus discharged. Cultures from this showed the development of a streptothrix after 4 or 5 days incubation. Microscopical examination of smears of the pus revealed no bacteria and were at the time regarded as negative, but on re-examination later delicate Gram-negative streptothrix filaments were observed. There was also oedema of the right foot and lower one-third of the tibia associated with localised tenderness.

17/1/35.—Leucocytes = 26,000 per c.mm., neutrophils = 95 per cent., lymphocytes = 2 per cent., monocytes = 3 per cent.

21/1/35.—A fluctuating subcutaneous abscess on the right leg was opened and about 1 ounce of thick, foul smelling, anchovy sauce pus was evacuated. Two out of six cultures showed colonies of streptothrix. Scarce mycelial elements were found in films from the pus.

The patient gradually became weaker and died in coma 3 days later (24/1/35) after a prolonged febrile illness lasting over three months (ninety-four days).

II.—POSTMORTEM EXAMINATION.

Autopsy was performed some 7 hours after death.

On opening the abdomen the liver was seen to be bound to the anterior abdominal wall and the edge projected 3 inches below the costal margin. There was a little clear fluid free in the peritoneal cavity, but no sign of generalised peritonitis. A mass was felt in the neighbourhood of the sigmoid flexure.

On separating the structures on the lower aspect of the liver a small quantity of thick green pus welled up from the lesser sac and the liver was bound down to an inflammatory mass in apposition to its inferior surface.

On sectioning the *liver* (1,580 grammes) multiple necrotic and suppurating areas were found, in one of which a branch of the portal vein was definitely thrombosed (*vide* Plate, Fig. 1). Foci of necrosis surrounded the portal vessels, extending widely into and destroying the hepatic tissues, while the pyaemic areas were occupied by glutinous, greenish pus which was odourless and contained no visible granules. Cultures were sterile as far as bacteria were concerned, but a streptothrix was again isolated.

The *sigmoid flexure* and the last few inches of the descending colon were matted together by inflammatory adhesions and bound down to the posterior abdominal wall. A few drachms of thick, glutinous, green pus exuded from the mucosa which shewed several punched out ulcers, one at least of which opened directly into the abscess cavity, while the remainder were more superficial and appeared to extend only to the submucosa. Diverticula were not found in this portion of the gut.

The hilum of the spleen was bound down to the tail of the *pancreas* and there was a small abscess involving both organs; it contained a small quantity of glutinous, greenish pus.

The *spleen* (265 grammes) except for the small abscess just referred to, was elsewhere free from disease.

The *stomach* and *upper intestine* contained a quantity of dark, tarry blood, the result of a large haemorrhage a short time before death, but the origin of the bleeding was not found.

The remaining viscera shewed no noteworthy morbid changes. The central nervous system was not examined.

Microscopic Anatomy.—There were wide areas of tissue necrosis and destruction of liver tissue with replacement by polynuclear and phagocytic cells and foci of abscess formation. Delicate filaments of streptothrix mycelium were seen ramifying in the necrosed areas and in the wall of the abscess cavities.

Here and there the mycelium was collected into tufts (Plate Figs. 2 and 3) in the form of a tangle of filaments and without any asteroid or ray formation and without clubs. The fungus was Gram-negative and not acid-fast so that it was difficult to identify amongst the necrosed tissues. It stained with haematoxylin, toluidin blue and other basic stains. In addition the parenchyma of the liver shewed numerous areas of cirrhosis surrounding the smaller bile ducts in the portal system which appeared as isolated, more or less circular, plaques. The bile ducts stood out prominently in these cirrhotic areas and were surrounded by polynuclear leucocytes, while their lumina were often plugged with these inflammatory cells. The cirrhosis was biliary in type and evidently of long standing, whereas the streptothrichal cholangitis was of more recent date. Liver flukes and ova were not demonstrable, but the type of cirrhosis resembled that seen in fluke infestation of the liver, and the possibility that it was the aftermath of a *Clonorchis sinensis* infestation remains.

Sections of the *sigmoid* shewed marked inflammatory hyperplasia of all the coats with some ulceration of the mucosa and necrotic tissue in the gut wall. Careful scrutiny of the necrosed area revealed the delicate filaments of a streptothrix infection. Granule and ray formation were absent as in the liver, whilst the mycelial elements were again Gram-negative and non-acid fast.

III.—CHARACTERS OF THE STREPTOTHRIX.

The streptothrix appeared as a delicate, unicellular, monomorphous mycelium shewing simple branching, older portions becoming segmented and assuming bacillary or granular appearances. Old fluid cultures shewed amorphous granules which developed into mycelium on fresh medium. Aerial hyphae were abundant on older cultures or on unsuitable media and shewed white powdery efflorescence. No conidia or spore formation occurred—unless amorphous granules are regarded as “spores.”

It grew freely in solid and fluid media aerobically, but under anaerobic conditions it grew feebly and only with difficulty, aerial hyphae not being produced, whilst the mycelial substratum broke up early into granules. It stained well with haematoxylin and the usual aniline dyes, was feebly Gram-positive, but decolorised readily and was not acid-fast though the mycelium withstood momentary application of 1 per cent. HCl.

The cultural characters and animal reactions were as follows :—

Broth.—Delicate “puff ball” growth at foot of tube. Later, flocculent cotton-wool wisps. No surface growth. Medium remained clear and limpid and not ropy. Old cultures shewed granular deposit only.

Agar.—It took 2 or 3 days to appear on first isolation, but grew more rapidly in subculture at 37° C. The colonies were circular with blurred edges, firm or hard, sinking into medium ; white efflorescence began in 3 or 4 days. Later,



FIG. 1.

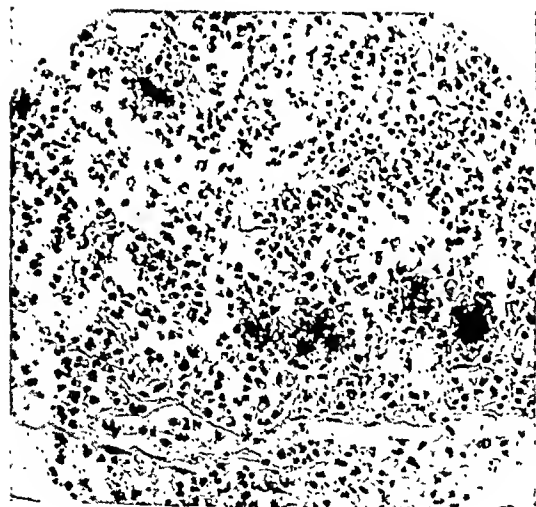


FIG. 2.

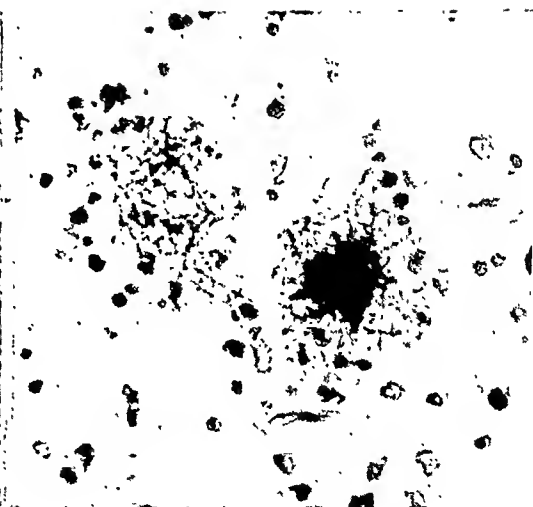


FIG. 3.

STREPTOTHRIX INFECTION.

FIG. 1.—Section of the liver shewing several areas of suppurative pylephlebitis with necrosis of tissue and the formation of small abscesses.

FIG. 2.—Section of necrotic tissue in the liver shewing the presence of several mycelial tufts ($\times 175$).

FIG. 3.—The same section shewing two mycelial tufts under higher magnification ($\times 600$).

Figs. 2 and 3 stained by haematoxylin and eosine.

the colony took on a dark centre by transmitted light: there was no colour formation.

Blood Agar and Serum Agar.—Growth more copious and efflorescence delayed for a week or ten days. No true haemolysis.

Gelatin.—Slow growth of formless wisp at room temperature. No liquefaction or clouding of medium.

Carbohydrate Media.—No change in lactose, glucose, mannite, dulcitol, sucrose, maltose, raffinose, galactose. Grew freely at the foot of the tubes and later climbed up Durham's tubes and shewed coarse pellicle and white efflorescence at surface. Medium remained quite clear. The fungus emitted a heavy musty odour from all media.

Potato.—Heavy growth and early white efflorescence. No colour change. Heavy mouldy odour.

Anaerobic Media shewed scanty circular cartilaginous colonies without lateral filaments or aerial hyphae. Growth was very poor.

Animal Experiments.—Rabbits, guineapigs and white mice were inoculated by the subcutaneous, intraperitoneal or intravenous routes and were killed at varying intervals up to 6 weeks. The animals remained healthy and cultures of the viscera were negative.

IV.—CHARACTERS OF AN ASSOCIATED ORGANISM.

This organism was found in some of the cultures from the pus either alone or associated with the streptothrix. It was a very small bacillus or coccobacillus, never filamentous, having a diphtheroid arrangement, almost Gram-negative except in large masses when it was sometimes Gram-positive. It was not acid-fast.

These characters remained constant and there has been no filamentous or mycelial formation. A large dose of the organism was given to a male guineapig by the intraperitoneal route, but without result.

V.—DISCUSSION.

Gram-negative diphtheroid bacilli have frequently been met with in association with actinomycotic infections and have been described by a number of observers including LIGNIÈRES and SPITZ (1904), particularly in relation to the group of actinobacillus. By some they have been regarded as a bacillary or coccial stage in the life history of the actinomyces or actinobacillus, but were this the case one would expect them to be capable of reversion to a filamentous type which has certainly not been the case in our cultures.

The streptothrix itself appears to be a representative of Group I, *Cohni-streptothrix* as described by ØRSKOV (1923) of which the type is *Actinomyces hominis* (Landsteiner) and which includes *A. madurae* (Vincent), *A. graminis* (Bostroem) and *A. rosaceus*.*

* We desire to thank Miss D. ERIKSON of the Lister Institute for her advice regarding the classification of this fungus.

VI.—SUMMARY AND CONCLUSIONS.

1. An acute streptothrichal infection of sudden onset, lasting 94 days, associated with chills, rigors, marked leucocytosis, enlarged tender liver and subcutaneous abscesses is described.

2. The primary lesion was in the colon, and, despite the colonic ulceration and peri-colonic suppuration found at autopsy, the faeces appeared normal to the naked eye and there were no clinical features suggestive of bowel trouble. In addition there was extensive suppurative pylephlebitis and an abscess involving the tail of the pancreas and spleen.

3. Blood cultures were negative, but a streptothrix was cultured from both arm and leg abscesses during life and from pus obtained from the liver at autopsy.

4. Filaments were found in films from the pus aspirated from pyaemic abscesses and they were demonstrated in tangled clumps in stained sections of the liver.

5. The cultural and other characters of the streptothrix have been studied ; it has been placed in Group I, *Cohnistreptothrix*, according to ØRSKOV's classification.

6. The pus during life and after death did not yield any of the ordinary agents of suppuration, but a small Gram-negative diphtheroid bacillus was present in several cultures.

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THE EFFECT OF LIGHT AND DARKNESS ON OVIPOSITION IN MOSQUITOES.

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INTRODUCTION.

In the summer of 1933, from the 19th June to the 10th July, there was a continuous laying of egg rafts by *Culex pipiens* on two ponds at the Laboratories, but one of these was utilised to a much greater extent than the other which was only five feet away. During the period mentioned about 80 rafts were laid on the first pond and only about 10 on the second. Investigation showed that the conditions of the water in one pond were not different from those in the other. Both ponds were alike as regards the colour of the background and of the water,

temperature and the pH, which varied from 8.0 to 9.0 ; neither was there any noticeable difference in their fauna and flora. It was evident therefore that any difference in the laying of the rafts was due to some factor outside the water.

The ponds are situated on a lawn, the first about 24 feet and the other about 35 feet from a high brick garden wall. Both are more or less equally exposed to the wind but they differ as regards illumination. The first, where the rafts were laid continuously, has a more shady position, owing to the proximity of a large tree, and it seemed obvious that the shade was the main factor which influenced the insects to favour this pond.

Since, in the natural conditions, it is impossible to study the effect of one factor without interference by the others, it was decided to arrange experiments in the laboratory in order to observe the influence of light and shade on the oviposition of this mosquito.

The literature on the influence of light on insects was reviewed by UVAROV (1931) and that on the same subject as regards mosquitoes by CHRISTOPHERS and CASSIROLI (1934). The only quantitative data on the effect of light and darkness on oviposition were given by BUXTON (1927), who found experimentally that darkness is more attractive than light to both *Aedes argenteus* and *A. variegatus*. Buxton's experiments were carried out on a verandah outside his laboratory in London. The experiments described in the present paper were made in the laboratory at Esher, Surrey.

EXPERIMENTS.

The experiments were carried out in cages each measuring one cubic foot. The main structure of each cage was a wooden frame, to one side of which was attached a sleeve of ordinary mosquito netting ; the other sides were covered with metal mosquito netting. As the humidity of the room was very low the cages were placed above large trays filled with water. Each week these trays were thoroughly washed and filled with clean water.

The trays with the cages were so arranged along the window that the side of each cage facing this window was equally illuminated. The arrangement of the cages with regard to the source of light is a very important point in experiments of this kind. It will be shown later that even a very slight shade on a part of the cage facing the source of light greatly affects the results. The space around the cages was also tested for draught, the absence of which was ascertained by watching the movement of tobacco smoke. For the laying of the eggs each cage was provided with two Petri dishes, which were filled with tap water or with one of the infusions mentioned below. The arrangement of the dishes inside the cage is just as important as the arrangement of the cages with regard to the source of light ; therefore the dishes of each cage were placed at the same distance from the side of the cage which faced the window. With this arrangement of the cages and the dishes it was possible to ensure that the latter received

the same amount of light, while owing to their proximity to each other and the absence of draught they were surrounded by air of the same temperature and humidity.

The experiments were made with the local *C. pipiens*, the Indian *C. fatigans* and the autogenetic race of *C. pipiens* discovered by ROUBAUD (1929)* and introduced into these Laboratories from Germany in 1932 by the late Dr. MACGREGOR. As the females of this mosquito produce fertile eggs without a blood meal, the opportunity was taken of studying the effect of light on oviposition in the fed and unfed insects.

The first experiment was arranged to determine whether the mosquitoes prefer a white or black background for oviposition. One of the dishes was therefore placed on a white and the other on a black paper disc. Both dishes were filled with tap water which, in order to ensure the same reaction, was changed every day. The experiment was carried on for more than ten weeks, during which time the pH of the water was 8.0. Into one cage were introduced females of the local *C. pipiens* and into the other fed females of the autogenetic mosquito. During the course of this experiment the local mosquito laid 62 rafts, 45 of this number being laid in the dish with the black background and only 17 in the other dish. The autogenetic mosquito laid 47 rafts, of which 41 were found in the dish with the black background and only 6 in the dish with the white background. The results obtained in this experiment show quite conclusively that the local mosquito and the fed females of the autogenetic race distinctly prefer dishes with a black background.

In order to test the effect of shade on the mosquito, one of the dishes in each cage was surrounded by a collar of black paper, the edge of which was about three-quarters of an inch above the edge of the dish. All the dishes were filled with tap water, which, as in the previous experiment, was changed daily. Into one cage was introduced the local *C. pipiens* while two other cages were used for the autogenetic mosquito, one cage for the fed and the other for the unfed females.

As regards *C. pipiens* the experiment gave an inconclusive result because with the advent of winter the mosquito stopped breeding in the laboratory. Its females laid only 11 rafts, of which 7 were found in the shaded dish. The experiment with the autogenetic race was however carried on for about 4 months during which period the fed females laid 349 rafts, 270 in the shaded dish and only 79 in the unshaded dish. Here it is again seen that the fed females of this mosquito preferred the shaded dish, in which they laid 54.7 per cent. more rafts. On the other hand the unfed females behaved more or less indifferently; of 935 rafts laid by them only 552, or 59.0 per cent., were laid in the shaded dish. It will be shown in another place that the unfed females of the mosquito

*It would seem that the use of the expression "autogenous" in this connection is not strictly accurate, accordingly the term "autogenetic" is employed throughout this paper.

also behaved more or less indifferently towards the different infusions which were used in these experiments.

Table I shows the results of a similar experiment with the autogenetic mosquito, where instead of tap water the dishes were filled with hay infusion. The pH of the infusion was 8.0 or 9.0 during the course of this experiment, but it was always the same in both dishes of the same cage. In this experiment, as in that where the dishes were filled with tap water, the fed females of this mosquito showed a distinct preference for the shaded dish, in which 38 of a total of 50 rafts were laid—a difference of 52.0 per cent. Here again the unfed females behaved almost indifferently. Of 332 rafts laid by them only 177, or 54.9 per cent. were laid in the shaded dish.

It is a well known fact that mosquitoes can lay their eggs in water of which the chemical conditions are absolutely unsuitable for the breeding of these

TABLE I.

Fed autogenetic <i>C. pipiens</i>				Per cent.
Shaded dish with hay infusion	38 rafts	76.0	
Unshaded ..	12	24.0	
Total 50 .. Difference				52.0
Unfed autogenetic <i>C. pipiens</i>				Per cent.
Shaded dish with hay infusion	177 rafts	54.9	
Unshaded ..	145	45.0	
Total 322 .. Difference				9.9

insects. Dr. COGHILL, in 1913, on the Gold Coast (see HANSCHALL, 1926) used to reduce the number of mosquitoes in the hospital by placing small pans containing weak copper sulphate solution in dark corners. The mosquitoes laid their eggs in these pans but the larvae died soon after hatching. SARMA (1921) also studied the influence of different mineral and organic substances on oviposition in the Culicidae. He compared the effect of these substances with water, but in his experiments the unequal illumination of the vessels was not taken into consideration. In the experiments of BUXTON and HOPKINS (1925), which were carried out in Samoa, the females of *Aedes argenteus* and *A. variegatus* laid their eggs in hay infusions which were toxic to the larvae owing to the presence in them of arsenous anhydrite or copper sulphate. But if the mosquitoes have a choice of different infusions they lay more eggs on that infusion the chemical conditions of which apparently most resemble those of the water of

their natural breeding places. It has also been shown by BUXTON (1927) that some infusions of organic matter are distinctly more attractive to mosquitoes than others.

In order, therefore, to test the effect of light against the attractiveness of one of the infusions, it was necessary first to find out what infusion is the most attractive to the fed and unfed autogenetic mosquito and to *C. fatigans*. In the following experiments the attractiveness of hay infusion was compared with that of tap water, dog biscuit infusion and infusion of leaves. The tap water was changed every day and the infusions every second or third day.

It is now customary to study the hydrogen-ion concentration (pH) of water during the investigation of the ecology of mosquitoes; however, after examining the results obtained by different observers it becomes obvious that unless the reaction of the medium is very acid or very alkaline the pH has not such a great influence on the breeding of mosquitoes as was formerly thought. BALLOWE (1918) found many larvae and pupae of *Anopheles* and *Culex* in a barrel containing the diluted solution of caustic soda used for killing the San José scale. The solution had been exposed to rain, but its alkaline reaction was marked. The data collected by SENIOR-WHITE (1934) show that *C. fatigans* can tolerate a pH between 6.5 and 9.6. This author also mentions an interesting case of the finding of larvae and pupae of this mosquito by his staff in a tank of diluted HCl, of which the pH was actually about 1.6.

The experiments with different infusions were carried on for about 6 weeks. During this period the pH of the water was 8.0. The pH of the hay infusion was 8.0 or 8.5, except for 2 days when it was 9.0, while it was being compared with the dog biscuit infusion, of which the pH was then 9.5. The pH of the dog biscuit infusion was 8.0 or 8.5, with the exception of 4 days when it was 5.5, 3 days when it was 9.0 and 2 days when it was 9.5. During these last 2 days it was being tested against the hay infusion of which the pH was 9.0, as already mentioned. The pH of the leaf infusion was 8.0 or 8.5. It is seen that the difference in the pH of the infusions was 0.5, with the exception of only 4 days, when it was 2.5. Therefore it is obvious that in the experiments the difference in the pH was not sufficient to produce any influence on these mosquitoes.

Table II shows the results of experiments with the fed and unfed autogenetic mosquito. In Cage 1, where the attractiveness of the hay infusion was compared with that of tap water, the fed females laid 212 rafts, 178 being in the dish with the hay infusion, *i.e.* 67.9 per cent. more than in the dish with the tap water. In the second cage, where the hay infusion was compared with the dog biscuit infusion, the fed females laid 173 rafts, 130 being laid in the hay infusion, *i.e.* 50.3 per cent. more than in the dog biscuit infusion. In the third cage, where the hay infusion was compared with the leaf infusion, the fed females laid 96 rafts of which only 53 were found in the hay infusion, *i.e.* only 10.5 per cent. more than in the leaf infusion. These results show quite clearly

that the hay infusion is the most attractive to the fed females of this mosquito. The table shows that it is also the most attractive to the unfed females which however do not display such a marked preference as do those that have fed.

TABLE II.

Fed autogenetic <i>C. pipiens</i> .				Per cent.
Cage 1	{ Hay infusion	.. 178 rafts	..	83.9
	{ Tap water	.. 34 „	..	16.0
Total		212 „	Difference	67.9
Cage 2	{ Hay infusion	.. 130 rafts	..	75.1
	{ Dog biscuit infusion	43 „	..	24.8
Total		173 „	Difference	50.3
Cage 3	{ Hay infusion	.. 53 rafts	..	55.2
	{ Leaf infusion	.. 43 „	..	44.7
Total		96 „	Difference	10.5
Unfed autogenetic <i>C. pipiens</i> .				Per cent.
Cage 1	{ Hay infusion	.. 532 rafts	..	73.2
	{ Tap water	.. 194 „	..	26.7
Total		726 „	Difference	46.5
Cage 2	{ Hay infusion	.. 132 rafts	..	65.6
	{ Dog biscuit infusion	69 „	..	34.3
Total		201 „	Difference	31.3
Cage 3	{ Hay infusion	.. 239 rafts	..	55.4
	{ Leaf infusion	.. 192 „	..	44.5
Total		431 „	Difference	10.9

Table III shows the results of the same experiments with *C. fatigans*. In Cage 1, where one dish was filled with hay infusion and the other with tap water, the females were attracted to the hay infusion almost to the same extent as those of the unfed autogenetic mosquito; of 151 rafts laid by them 106 were found in the hay infusion, *i.e.* 40.3 per cent. more than in the tap water. In

Cage 2, where one dish contained hay infusion and the other leaf infusion, the insects laid 245 rafts, of which only 134 were found in the hay infusion. In this experiment they were again attracted to the hay infusion in the same degree as those of the unfed autogenetic mosquito. In Cage 3 they showed no discrimination between the hay and the dog biscuit infusions, in both dishes they laid practically the same number of rafts.

It has been shown in Table II that the fed females of the autogenetic mosquito laid 10.5 per cent. more rafts in the hay infusion than in the leaf infusion. This result was obtained when the dishes of the cage were equally illuminated. In order to find out the effect of unequal illumination on the oviposition in these two dishes, the cage was placed near the side of the window, half of the wall of the cage being opposite the wall of the room, the other half opposite the window and therefore better illuminated. In the cage so arranged,

TABLE III.

<i>Culex fatigans</i> Wied.					Per cent.
Cage 1	{ Hay infusion	..	106 rafts	..	70.1
	{ Tap water	..	45 „	..	29.8
		Total	151 „	Difference	40.3
Cage 2	{ Hay infusion	..	134 rafts	..	54.6
	{ Leaf infusion	..	111 „	..	45.3
		Total	245 „	Difference	9.3
Cage 3	{ Dog biscuit infusion	77 rafts	50.3
	{ Hay infusion	..	76 „	..	49.6
		Total	153 „	Difference	0.7

the dish with the hay infusion was placed in the shaded half and that with the leaf infusion in the illuminated half. The fed autogenetic mosquito laid 71 rafts in this cage, 52 being found in the hay infusion, that is 46.5 per cent. more than in the leaf infusion. The increase in the number of rafts by 36.0 per cent., as compared with the result obtained when the dishes were equally illuminated, is accounted for by the more shaded position of the dish. In the next experiment the dishes were reversed. The dish containing the leaf infusion was placed in the shaded part of the cage while that with the hay infusion was in the illuminated part. During the course of this experiment 156 rafts were laid, only 67 rafts being in the dish with the hay infusion, the rest, or 57.0 per cent., were found

in the dish with the leaf infusion ; that is 14.1 per cent. more than in the hay infusion which the females generally prefer when both dishes are equally illuminated, as has already been shown in Table II, Cage 3.

The same experiment was also made with tap water and hay infusion, since the greatest difference in the count of rafts had been obtained when the attractiveness of these two infusions was tested under equal illumination. In this experiment the cage was placed wholly against the window so that it was evenly illuminated. The dish containing the tap water was shaded by a collar of black paper, as described in one of the earlier experiments, while the dish with the hay infusion was left open. In this cage the autogenetic mosquito laid 192 rafts, 43 being found in the tap water and the remaining 149 in the hay infusion, *i.e.* 55.3 per cent. more in the hay infusion. On comparing this difference with that obtained under equal illumination (Table II, Cage 1) we find that it is smaller by 12.6 per cent. The decrease in the number of rafts in the hay infusion and their increase in the dish with tap water was produced by the shaded condition of the latter dish.

CONCLUSION.

These experiments confirm the observations of BUXTON (*loc. cit.*) and show quite clearly that light has a very great influence on the behaviour of the mosquitoes. It is a factor which must always be kept in mind when studying the influence of other factors on these insects.

The results of the first two experiments in which the effect of white and black backgrounds were studied and those where the attractiveness of the hay infusion was compared with that of leaf infusion under different illuminations are even more convincing, because in these experiments the landing conditions were exactly the same as the dishes were not surrounded by a black collar. The last two experiments show also very clearly that mosquitoes may lay more egg rafts in water of which the chemical and some other conditions are not as attractive to them as the water in the other dish provided the light factor is more favourable near the surface of the less attractive water. This is again in agreement with the observations on the oviposition of the local species of *Culex pipiens* in the ponds near the Laboratories, as described in the introduction to this paper.

In the course of these experiments the mosquitoes laid 4,583 rafts, of which number only 3 were found during daylight—one about half an hour before sunset and the other two about nine o'clock in the morning. Immediately after oviposition the egg raft has a white or slightly creamy-white colour. In daylight the eggs require about 5 hours to develop their black pigmentation. As no other unpigmented rafts were found after 9 o'clock in the morning or before complete darkness in the evening, it is safe to assume that all the other rafts were laid at night. This shows that in mosquitoes the phototactic response

must be very acute, since under a low intensity of light the difference in the illumination of the open and the shaded dish is greatly reduced, while in complete darkness there will of course be no difference at all.

In this connection it may be useful to mention that other experiments show quite conclusively that mosquitoes can develop perfectly, mate and lay fertile eggs in complete darkness.*

In the complete absence of light mosquitoes are neither positively nor negatively phototactic because there is no stimulus to develop the reaction, which can only occur when a difference in the illumination exists; therefore, in complete darkness, oviposition is governed either by the chemical condition of the water, or of the water vapour which is given off from the dishes. or by the combined action of these two factors.

The experiments also show that the tactic reactions of the mosquitoes are greatly influenced by the blood meal, since in all the experiments with the autogenetic mosquito the unfed females showed less marked preference in regard both to the different infusions and to illumination than the fed ones. From this may be inferred that in the natural condition the unfed females of this mosquito can utilize a much wider variety of water for oviposition than the fed ones.

In *C. fatigans* the preference is even less distinct than in the autogenetic mosquito, as shown in Table III. The results of this experiment agree with the observations made by SENIOR-WHITE (*loc. cit.*) in India, where the females of this mosquito utilize almost every variety of water for oviposition.

SUMMARY.

The experiments were made with two races of *Culex pipiens* and the Indian *C. fatigans*.

The results of the experiments show quite conclusively that just before oviposition the mosquitoes become more attracted to darkness than to light.

Almost all the egg rafts (4,583) were laid at night, when the difference in the illumination of the shady and of the open dishes was greatly reduced. This shows that the phototactic reaction in mosquitoes is very acute.

The autogenetic mosquito can develop perfectly, mate and lay fertile eggs in complete darkness.

In complete darkness oviposition is governed either by the chemical condition of the water or water vapour, or the combined action of these factors, because the stimulus producing the phototactic reaction is absent.

Of all the infusions the mosquitoes prefer that of hay, but they may lay more rafts in other infusions exposed to more favourable light conditions.

The tactic reactions of mosquitoes are greatly influenced by the blood meal.

*The results of these experiments will be published in another paper

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HYPERENDEMIC MALARIA IN A NATIVE RESERVE OF KENYA AND THE INFLUENCE UPON ITS COURSE OF ATEBRIN AND PLASMOQUINE.

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The primary object of the investigation was to determine the efficacy as an anti-malarial measure of small doses of atabrin and plasmoquine administered to the inhabitants of selected villages in a native reserve where anti-anopheline measures are largely impracticable. Experiments in control by medicaments alone is one of the recent recommendations of the League of Nations Malaria Commission (1934) in their programme for future malaria research. Various circumstances rendered the present enquiry peculiarly suitable for such a research: the reserve selected is small and compact and has been subject to considerable investigation in the past, the conditions are much more exacting than many of those pertaining elsewhere in that the inhabitants live a primitive, uncontrolled existence and finally, because of this, the results could be applied in other native areas, with a much greater certainty of repetition than those of investigations in highly disciplined labour lines, barracks, towns, etc.

Mass control with these drugs has chiefly been applied to the labour forces of estates and taken as a whole the degree of success has varied in direct proportion to the efficiency of the control exercised; when this has been considerable, morbidity due to malaria has been much diminished.

The second object of the investigation was to make an intensive study of the conditions relating to hyperendemicity, particularly to the course of infection and fever in individuals, changes in species, gametocyte production, and infantile mortality due to malaria. It was felt that the experiment should be conducted upon an "individual" basis and that each individual and his blood-slide should be examined by the writer personally once a month and by the African assistant at least ten times during the month so that the actual malaria morbidity in

relation to parasite rates could be more closely followed than is usually the case in large field investigations of a more purely statistical nature. For this reason, the number of people had to be strictly limited and the length of observations was confined to a year.

Hyperendemic malaria as a rule shows little seasonal variation, being affected, in fact, practically only by very abnormal rain, famine or non-immune immigration; and during the period of this experiment, conditions were particularly stable owing to deficient rainfall.

LOCALITY AND INHABITANTS.

Taveta was the place selected for the experiment. It lies near the Tanganyika border of Kenya Colony, about 3° south of the Equator and at an altitude of 2,500 feet. The native inhabitants live in a strip of forest land, about 9 miles long by 4 wide along the banks of the River Lumi, which rises a few miles outside the reserve in the "bottomless" crater lake Chala. At the opposite end of the reserve, the river debouches into the swamps of Lake Jipe and thence by way of the Rufu to the Pangani and Indian Ocean. Its waters and those of its tributaries play an important part in the malaria of the locality. Numerous fountains and springs in the forest supply the largest volume of water as it is only here, where the laval strata have petered out that the icy water flowing hitherto underground from Kilimanjaro is able to emerge to the surface. In this area, therefore, the water table is exceedingly high in comparison with the laval stretches between it and Kilimanjaro, as shown in the following table :—

TABLE I.
DEPTH AT WHICH WATER IS REACHED IN WELLS.

Place.	Depth in Feet (in Dry Season).
<i>Villages in Forest</i>	
Msengoni	6
Mbogoni	5½
Selandongo	3
<i>Just outside Forest</i>	12
<i>One mile from Forest</i>	40

The constant large supply of water from underground, allows the people to make use of it in irrigation channels and these form, even more than the river itself, the source of the anophelines.

Taveta was uninhabited until 300 years ago, when continual streams of natives of all tribes within a radius of 100 miles or more of Kilimanjaro invaded the forest and eventually intermingled to form the present Taveta tribe. Even to-day, natives of Pare, Masai, Ukamba, Taita, Chagga, etc., are immigrating to the fertile paradise of Taveta from their own drought- and famine-stricken lands, and this often non-immune immigration possibly plays a part in keeping the malaria continuously effervescent. Taveta in early days was a place of importance; it was an important halt on the great caravan route to the Chagga Kingdom, it was a Church Missionary Society and Administrative Station and it was the place where the first shot was fired in the Great War in East Africa. It has lost all its importance now, however; it is no longer a Government post, the mission is moribund and the natives themselves are probably little different from their ancestors with the exception of the abolition of a few of their more barbarous customs, *e.g.* killing of twins. They are a decadent race but with the decadents' ironical toleration of welfaring interference; and, for this reason, the regular administration of drugs, taking of blood-slides, temperatures, etc., was fairly easily carried out. A few local customs and habits gave rise to minor difficulties, most of which could be obviated for the term of the experiment. For instance, in the rare instances when families suffer much from fever they move to other localities to "try the air." Also an atavistic nostalgia for the mountainous countries of their origin leads the inhabitants to take frequent week-ends in Pare, Kilimanjaro, etc. As a rule, it was possible to make up their atebirin and plasmoquine dosage on their return. An inconvenient custom is that of changing their names, a year or so after birth and again after circumcision, and the refusal of a newly-wed wife to utter or allow to be uttered her name for the first month after marriage.

The population in 1933 was approximately 3,000. Malaria and drink are alleged to be the chief causes of the tribe's deterioration, but it is doubtful, as KAUNTZE and SYMES (1933) point out, how much ill-health is caused by the malaria and even the high figure of infantile mortality (57.1 per cent.) obtained by ANDERSON (1929) by interrogation was not confirmed by the actual case histories of infants in the present investigation. The spleen rate of children of Mbogoni, during the years 1927-1930, varied between 64 per cent. and 97 per cent (84 per cent. in June, 1930) and the parasite rate between 50 per cent. and 82 per cent. (63 per cent. in June, 1930). Blood slides (thick drop) of forest children taken by Dr. D. BELL and J. HARPER and examined by the writer at the Medical Research Laboratory, Nairobi, in 1930 showed the following variations:—

1930.	Percentage infected.	1930.	Percentage infected.
March	54	November	55
June	63	December	82
August	59		

The fact that the same children were not consistently examined each month makes exact comparison impossible. Quinine distribution to people suffering from fever in the reserve was carried out during the whole of the above period and up to the time of the commencement of the present experiment, but no change in the parasite or spleen rates resulted.

ADMINISTRATION OF ATEBRIN AND PLASMOQUINE.

Three villages or sub-locations were chosen for the experiment, *viz.*, Mbogoni for the atebirin test, Selandongo for the plasmoquine and Msengoni as a control. They were selected as far as possible to conform with the following requirements: comparative isolation from each other, situation entirely in the forest and a general similarity of conditions such as proximity to the Lumi River, etc.; the control village appeared to be the least mosquito-infested and had the fewest irrigation canals. The following average number of children and adults in each village were present each month from August, 1933, to September, 1934.

TABLE II.

Village.	Average Number per Month.	
	Children.	Adults.
Mbogoni	20	26
Selandongo	23	25
Msengoni	17	23

The numbers varied slightly each month, owing to emigration and immigration, births and deaths, etc., but practically the same people were examined throughout the period.

*Dosage of Drugs.**

One gramme of atebirin was given monthly (from September, 1933, to August, 1934, inclusive) to each adult of Mbogoni and proportionately less for children, in ten separate doses of 0.1 gramme and a total of 0.1 gramme of plasmoquine similarly to the inhabitants of Selandongo. The drugs were administered by a reliable African assistant, who visited each hut in turn on the 20 specified days every month and recorded the amounts taken and the date.

* Atebrin and plasmoquine simplex were kindly supplied for the first half of the experiment by the Pharmaceutical Department of Messrs. Bayer, Meister Lucius, and for the second half by the Medical Department of the Kenya Government.

The value of the experiment of course depended upon the constancy of this administration ; surprise visits to Taveta, checking of the records, questioning of the inhabitants, etc , never once revealed any delinquencies. The urines of the atebirin group were tested on several occasions, but neither the test recommended by the manufacturers nor that of GREEN gave satisfactory results ; even the urine of patients, treated in hospital with full courses of atebirin, failed to show colour changes or fluorescence which could be distinguished with certainty from normal urine controls—such was likewise the experience of DUNCAN (1934) in Singapore.

MEASUREMENT OF RESULTS.

The amount of malaria in any place can be regarded from two different points of view—firstly and most naturally, the amount of morbidity which it causes and which is measured by the number of attacks of fever (and deaths) from which the inhabitants suffer and secondly, the distribution of the parasite in man and in the mosquito. In epidemic malarious regions, these two indices reflect each other and correspond, but in a hyperendemic area, their relationship is inconstant and ill-defined and parasite rates here are only of direct value for the estimation of the potential danger of the place to immigrants. Parasite rates in hyperendemic areas give a picture of the plasmodial situation rather than the malarial.

In this experiment four criteria have been used :—1. Parasite rate ; 2. Spleen rate ; 3. Parasite intensity ; 4. Attacks of fever.

Parasite Rate.

This was determined monthly by taking blood-slides (thick drop) from the inhabitants of the three villages. The slides were stained with Giemsa stain.

Spleen Rate.

Palpable enlargement of spleens in children and adults were recorded at the beginning and end of the experiment.

Parasite Intensity.

The number of parasites per 50 fields of a thin blood-smear, stained with a modified Gordon's stain, were counted at the beginning and end of the period and the results are expressed as the average number of (sub-tertian) parasites present in the bloods (positive and negative) of the children and adults respectively in each locality.

Fever.

The African assistant made careful enquiries, during his tour of the villages when distributing the drugs, about attacks of fever amongst the inhabitants.

The people of Mbogoni and Selandongo were examined thus at least ten times during the month and those of the control village, Msengoni, ten times also. "Fever" cases were then only recorded if they complied with the following:—

1. Temperature higher than normal. (If a case with a normal temperature was strongly suspected of being malaria, the assistant returned in the afternoon to take the temperature again).

2. Absence of symptoms of coryza, influenza, bronchitis, etc. Measles and whooping cough were easily distinguished and excluded.

The number of attacks of fever were then averaged out as number per year for child and adult in each sub-location. The adult fever figures cannot be compared with those of the children, as the latter probably have more fever than is complained of and recorded.

The results of the above indices are shown in the accompanying graphs and tables.

TABLE III.
SPLEEN RATES.

Place and People.		August, 1933. Percentage.	September, 1934. Percentage.
<i>Mbogoni</i> (Atebrin)	Adults	47	50
	Children	82	74
<i>Selandongo</i> (Plasmoquine)	Adults	27	33
	Children	43	62
<i>Msengoni</i> (Control)	Adults	63	60
	Children	50	53

TABLE IV.
PARASITE INTENSITY.

(Average number including negatives, of sub-tertian parasites per 50 fields.)

Place and People.		August, 1933.	September, 1934.
<i>Mbogoni</i> (Atebrin)	Adults	$\frac{1}{4}$	$\frac{1}{2}$
	Children	10	6
<i>Selandongo</i> (Plasmoquine)	Adults	$\frac{1}{2}$	$\frac{1}{4}$
	Children	27	6
<i>Msengoni</i> (Control)	Adults	$\frac{1}{2}$	$\frac{1}{3}$
	Children	1	12

TABLE V.
ATTACKS OF FEVER.
(Average number per individual per year.)

Place.	Adults.	Children.
<i>Mbogoni</i> (Atebrin)	1.3	2.0
<i>Selandongo</i> (Plasmoquine)	0.8	1.0
<i>Msengoni</i> (Control)	1.1	1.1

The Result of the Experiment.

Before attempting to estimate the actual results, it is as well to consider the possible action of the drugs in the present circumstances :

The plasmoquine has two possible effects :—

1. Personal prophylaxis. The dosage is much too small to be expected to have any real value in this connection ; it is known that even large daily doses of the drug do not give uniform success.

2. Crescent steriliser. With similar doses to those employed in the present experiment, WALLACE (1933) in Malaya was able to cause a reduction in the malaria amongst estate labourers and BARBER, RICE and BROWN (1932) obtained a marked diminution in the sporozoite index in a forest plantation in Liberia.

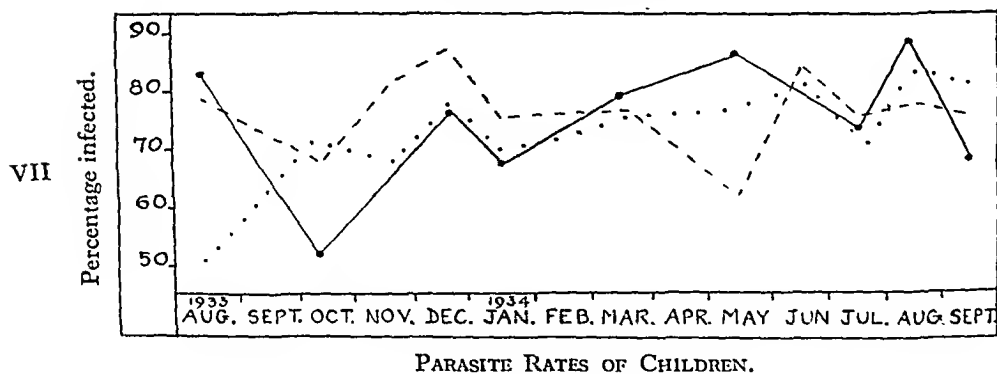
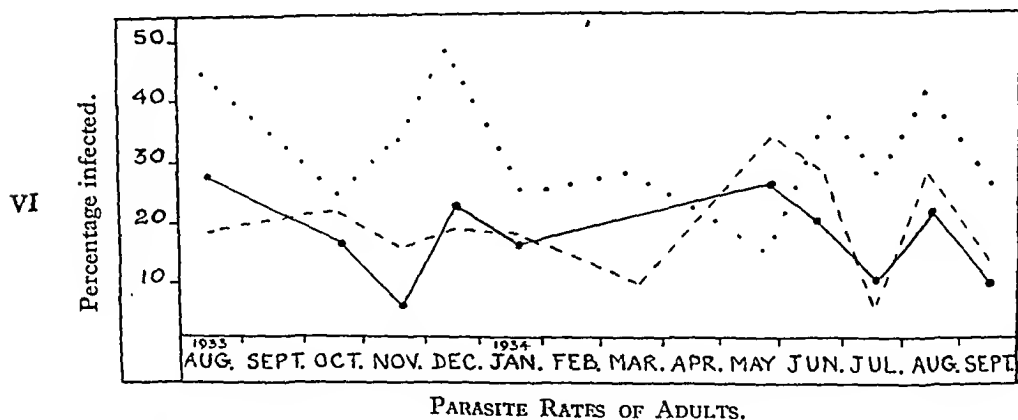
The atebrin also has two possible effects :—

1. Curative. The dosage, though small, is likely to have a therapeutic value, as the drug is excessively cumulative.

2. Atebrin, though probably not a specific "sporozoiticide" has a prophylactic action like quinine and is able to keep infections sub-febrile.

The sole visible result of the experiment was the fairly well-marked diminution in parasite intensity in the treated areas as compared with the control village. (See Table IV, page 172). The numbers of parasites in Mbogoni and Selandongo inhabitants were roughly halved by the end of the administration of the drugs, whilst those of the Msengoni children showed a marked increase. Such a result is the well-recognized aim of anti-malarial measures in Europe, but these have been applied chiefly to endemic and epidemic malarious localities, where the results are accompanied by a diminution in the intensity and number of malaria attacks. As I have already pointed out, in a truly hyperendemic area, malaria, as a rule produces so few symptoms that any abatement corresponding to a reduction in the parasite intensity is unnoticeable.

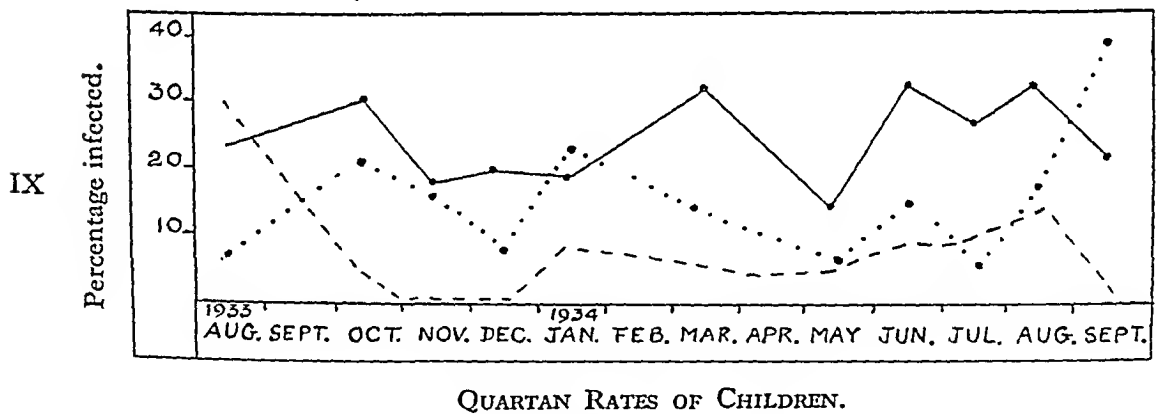
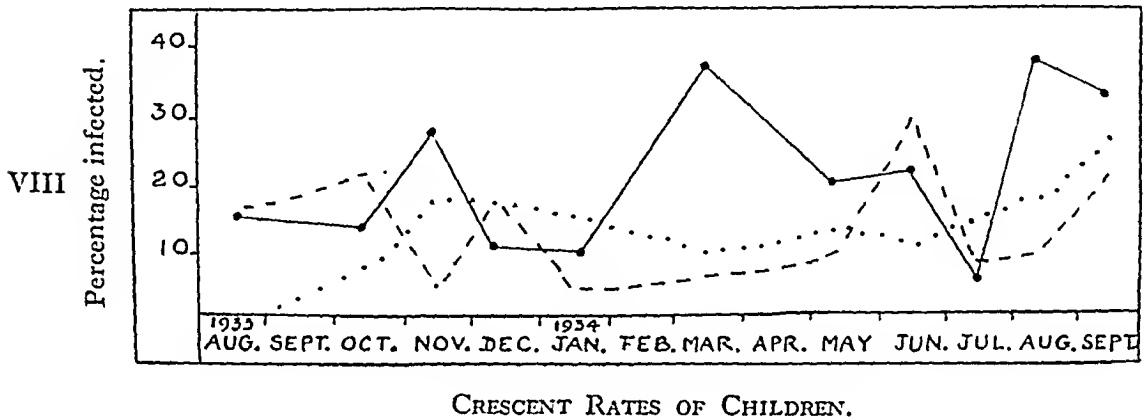
The parasite (Table VII) and crescent (Table VIII) rates in children show no differences of any significance in the three areas and an examination of the specimen protocols (*e.g.* Protocol 7, p. 186) reveals the fact that fresh infections are in no way prevented by the use of these drugs. In the case of atebtrin, one is rather forced to the conclusion, that it (like, in all probability, quinine) has an effective action in malaria only during the pyrexial periods and therefore, particularly in a hyperendemic area, where fever is the exception rather than the rule,



— = Mbogoni (atebrin); - - - = Selandongo (plasmoquine); . . . = Msengoni (control).

no change in the situation after atebtrinisation, will be observable. It is possible in the present experiment that the failure of plasmoquine to prevent the contraction of new infections was due to the insufficient isolation of the area concerned though it was separated by half a mile or more, from untreated villages. Also the presence of a single infective person for one night only would suffice to infect many anophelines and thus ruin the experiment. These defects are, of course, the major disadvantages to anti-malarial schemes based on drug control for people living in a natural environment, as compared with specially cited and disciplined labour forces.

The curves of the total parasite rates for both children and adults (Tables VII and VI) show certain fluctuations but there is no sign whatever of a steep drop in the curves relating to the treated villages during the course of the experiment. The greater irregularity in the adult curves as compared with those of the children merely represents the effect of using only a small number of lightly infected people: in larger groups of people (or, in more heavily infected groups, as occurs in the children), individual variations would be more thoroughly



— = Mbogoni (atebrin); - - - - = Selandongo (plasmoquine); . . . = Msengoni (control).

averaged out. The point to be realised is that the variations are largely haphazard and bear no relation to the administration of the drugs.

THE COURSE AND NATURE OF HYPERENDEMIC MALARIA.

Clinical Features.

The mild character of the malaria in the indigenous inhabitants of a hyperendemic region is surprising when it is remembered that if Europeans enter it—as occurred at the beginning of this century in the case of Taveta—many will

die of cerebral malaria or blackwater fever. Immunity is of course the explanation in adults, but it is difficult to understand why infants also suffer so little. The survey of infants, which will be described later, showed that in spite of heavy and 100 per cent. infections often with the three species of parasite, the children were well-nourished and healthy-looking. Even deaths from malaria in infants were apparently due as much to a co-existent anaemia or diarrhoea as to malaria itself and just as no schizonts of *P. falciparum* were discovered in their bloods, no cases of cerebral or fulminant malaria were reported. Death on the other hand, from benign tertian malaria, which is so rare in Europeans occurs in the natives of this area. (E.g. Two infants of 3 and 4 months respectively died as a result of heavy infection with *P. vivax*—the latter only being an inhabitant of an experiment village—see Protocol 7, page 186.)

Approximately a third of both children and adults appeared to have no fever throughout the period of observation, in spite of heavy malarial infection (in the former) and Table V shows that the average amount of fever per person per year is little more than one attack. It is probable that these figures do not include all slight attacks, though the constant visits to the villages are bound to have revealed all the crippling ones. In order to verify the absence of fever (though it must be remembered that the investigation was confined to complaints of "fever" and not pyrexia) the temperatures of four children were taken twice daily for a week. None of the children during this period had a temperature reaching 100° F., though all showed slight rises at one time or another, but at these times they did not complain of feeling feverish.

Protocol 1 (Mb. 28), on page 183, gives an example of the mild character of the malaria in an infant and Protocol 2 (S. 48) shows a similar case but with absence of fever. In Protocol 3 (M. 43A) it is seen that even the contraction of its first infection by a new-born child was unaccompanied by fever. The reverse side of the picture is provided by cases such as are illustrated in Protocol 4 (Mb. 38) in which pyrexia is common but the presence of parasites infrequent.

Infantile Mortality.

It is, of course, impossible to give a definite figure of this index, based upon the small number of infants examined in the investigation, but it is interesting to see what happened to them during the year when 4 died out of the total of 10 examined.

In only one of these cases (that of the triple infection) can death be directly ascribed to malaria; in two, it was probably a contributory factor and in the first probably quite incidental.

No deaths from malaria in older children or adults occurred during the period of the investigation.

TABLE X.

Age at Death.	Cause of Death.	Number of Months with Malaria.
8 months	Broncho-pneumonia following measles	3 months
4 "	Diarrhoea and heavy B.T. malaria (Protocol 7)	1 month
11 "	S.T., Q. and B.T. malaria	3 months
5½ "	Intense anaemia with scanty S.T. malaria (less than 1 parasite per 50 fields)	1 month

Age Incidence of Infection.

The relationship of parasite rates to age showed at Taveta the usual features associated with hyperendemicity—an abrupt rise in infancy to a 100 per cent. infestation, followed by a slow decline through childhood and adolescence to roughly a third infected in young adults (as at Msengoni, where there was a majority of this group—see Table VI) and a quarter in maturity (as at Selandongo and Mbogoni). Of more importance than this general wave, typical of hyperendemicity, is that provided by parasite rates in infants per month of age and named by SCHILLING and NEUMANN (1933) "New-Born Index" (an ambiguous designation, since it suggests congenital malaria).

TABLE XI.

PERCENTAGE OF INFANTS INFECTED ACCORDING TO AGE.

Age.	Number Examined.	Parasite Rate.	Sub-tertian.	Crescents.	Benign Tertian.	Quartan.
1 month	2	0	—	—	—	—
2 months	3	33	33	33	—	—
3 "	3	66	33	33	33	—
4 "	5	20	20	20	—	—
5 "	7	86	57	43	14	29
6 "	4	50	50	25	—	—
7 "	4	100	100	100	—	75
8 "	3	100	100	33	—	66
9 "	2	100	100	50	—	—
10 "	5	100	100	80	—	20
11 "	9	100	100	44	11	89
12 "	20	100	90	45	15	85

As pointed out by HACKETT the age of onset of the first attack provides an ideal "primary index" of the malariousness of the district. Table XI shows

the infections per month of age in a group of 67 infants in the Taveta forest examined at the end of the dry season. It is seen that during the first 6 months of life, a few infants manage to escape infection, but that after that age, 100 per cent. are infected. More specific dates of first infections are provided by the infants born in the experiment villages and who were examined every month. They became infected as follows :—

TABLE XII.

Date of Birth.	Date Infected.	Species.
2.1.34	8.9.34	<i>P. falciparum</i>
15.2.34	12.6.34	<i>P. vivax</i>
7.3.34	15.8.34	<i>P. falciparum</i>
13.3.34 (Protocol 3)	12.6.34	<i>P. falciparum</i>

Both *Plasmodium vivax* and *P. falciparum* appear at the end of the second month of life, but infections of *P. malariae* are delayed—presumably because of the extremely long incubation period of this species—until the fifth month. Towards the end of the first year, however, quartan infection becomes well-established, nearly all the infants, a year old, being infected with this parasite; later in childhood its incidence lessens and in nearly a thousand examinations of adults there were only 1.5 per cent. of quartan infections.

Approximately half of the infants of all ages (after the second month) had crescents in their blood; the gametocyte incidence diminishes later in childhood as is indicated in Table VIII and in adults crescents are rarely found.

Species and Morphology of the Parasite.

The prevalent species and the only one that persists into adult life is *P. falciparum*. The quartan parasite is hardly less common in young children, but rapidly disappears with increasing age. In other words, as THOMSON (1934) has recently pointed out, immunity towards quartan is established much sooner than to sub-tertian (see Table XI for quartan infections in infants and Table IX for those in older children). *P. vivax* is only occasionally encountered. The sub-tertian parasite is ubiquitous as regards locality and *P. malariae* only slightly less so, though it occasionally shows a more focal distribution, e.g. a few families will be persistently negative for this parasite throughout the months of examination, whilst others in the vicinity and of a similar age constitution will show the usual quartan infection in the young children. Benign tertian malaria, on the other hand, is markedly localised in distribution, for instance in the 67

children of 1 year and under (Table XI), the benign tertian cases (with one exception) all came from a single village.*

Seasonal changes in the parasite rates were not particularly noticeable (see Tables VI and VII, page 174, and Table XIII for rainfall and temperature).

TABLE XIII.

RAINFALL AND AVERAGE MAXIMUM AND MINIMUM TEMPERATURE.

Month.	Rainfall in Inches.	Maximum Temperature. (°F.)	Minimum Temperature. (°F.)
Aug., 1933	0.34	80	65
Sept. "	0.00	82	65
Oct. "	0.13	89	68
Nov. "	1.12	91	70
Dec. "	1.92	93	70
Jan., 1934	0.27	95.5	69.5
Feb. "	0.66	97	71
Mar. "	0.79	92	72
Apr. "	2.50	89	71
May "	4.30	80	72
June "	1.27	77	65
July "	0.33	79	64
Aug. "	0.00	80	66
Sept. "	0.15		

What appeared to be new contractions of quartan malaria were most common in August, 3 months after the rainy season and apparently new infections of benign tertian were commonest in the month of June, *i.e.* 1 month after the rains. The fleeting appearance of *P. vivax* and to a lesser extent of *P. malariae* makes it difficult to diagnose fresh infections with certainty. Frequently benign tertian parasites appear for a month and are not seen again or perhaps not until 8 or 9 months later; often their presence seems to oust temporarily a previously persistent sub-tertian infection (see Protocols 5 and 6, pages 185 and 186).

There seems likewise to be little seasonal fluctuation in the crescent rates, though there is a suggestion of an increased production during the dry seasons (*e.g.* March and August in Mbogoni, see Table VIII). A hyperendemic area, such as Taveta, is in this way in marked contrast to districts subject to fulminant

* One is constantly meeting with examples of localisation of benign tertian malaria, *e.g.* the only cases of this infection discovered in the small town of Voi (75 miles from Taveta) during the same period as that of the experiment, occurred simultaneously in two Indians living in a single room of a Railway landhie and the only cases of benign tertian, contracted by prisoners in Fort Jesus, Mombasa, in 1932, occurred within a day of each other in occupants of adjacent cells.

epidemics, *e.g.* Northern Sind in India, where COVELL and BAILEY (1933) reported an abrupt rise in the crescent rate about the ninth week of the epidemic.

The morphology of the sub-tertian parasites corresponded to the appearances described by the writer (GARNHAM, 1933) as characteristic of an East African (?) sub-species of *P. falciparum*. Certain infants in their initial attacks of malaria showed these features (*e.g.* large, solid or tenuiform parasites in enlarged cells with Maurer's dots well-marked), thus demonstrating that the peculiarities are not—as has been suggested—the result of long-continued infection.

The quartan parasites presented the following atypical (or little described) appearances :—

1. Tenuiform rings common.
2. Large vacuole in two-thirds grown trophozoites.
3. A well-defined peripheral thickening (ectoplasm or capsule) in the half to three-quarters grown forms (GARNHAM, 1933).
4. Delay in the division of the chromatin until a few hours before breakage of the schizont.
5. The pigment often golden yellow rather than black.

ANOPHELINES AT TAVETA.

During the period of the experiment *Anopheles gambiae* (*costalis*) was the predominant house-haunting mosquito, whilst *A. funestus* was much less frequently found. Daily searches and counts were not made, but the following figures give a rough idea of the proportions of the two species :—

TABLE XIV.

ANOPHELINES CAPTURED IN HOUSES IN THE THREE EXPERIMENT VILLAGES.

Month (1934).	<i>A. gambiae.</i>	<i>A. funestus.</i>
January	65	8
February	60	14
March	42	8
April	74	18
May	99	10
June	67	4
July	39	5
August	Nil.	Nil.
September	"	"

The following anophelines were also caught on rare occasions as adults in huts :—*A. pretoriensis* ; *A. mauritanus* ; *A. marshalli* ; *A. natalensis*.

The following species of *Anopheles* were captured in larval form :—*gambiae* ; *marshalli* (at least 2 varieties) ; *pretoriensis* ; *natalensis* ; *transvaalensis* ; *longipalpis* ; *mauritanus*.

KAUNTZE and SYMES (1933) found both *A. gambiae* and *funestus* infected with malaria in Taveta, the former species up to 6·3 per cent. and undoubtedly these two mosquitoes are the chief agents responsible for the transmission of the disease, though it is possible that *A. marshalli* also plays a part, as this species has been found naturally infected in other parts of East Africa. In the present investigation, no special seasonal predominance of one or other species was noticed and there was no particular correlation between the anophelines and fever or parasite rates.

DISCUSSION.

From the above study it appears that the aim of anti-malarial measures in a hyperendemic area must be in a very different direction from the aim of those in epidemic or endemic regions. In endemic, we want to reduce the amount and severity of fever and have succeeded in doing so sometimes by drugs ; in epidemic, to prevent the occurrence of malaria ; but in a hyperendemic, what is to be our objective ? Here, it is a question not of prevention, but of eradication, which, *sui generis*, must usually be a task of huge magnitude, and not a question of diminishing the amount of fever, because there is no fever or very little, except when the balance between immunity and parasites is upset.

There would appear to be a certain similarity between the hyperendemic malaria of tropical Africa and the malaria which occurs in monkeys, reptiles, wild birds, etc., in many parts of the world. As in birds or monkeys, but probably not quite to such a degree, immunity has become so complete that morbidity is reduced to a minimum. This appears to apply only to subtertian and quartan malaria ; with benign-tertian, infections appear to be much more virulent, possibly owing to the later introduction of this species and consequent lesser degree of racial immunity. A certain amount of morbidity must result in man and animals from the destruction of erythrocytes—a necessary concomitant of the parasite cycle, but possibly after centuries of this, blood destruction of this type hardly differs in its effects from the blood destruction which occurs as a normal event in the body's mechanism.

It is seen that the administration of drugs had no effect beyond a reduction in the parasite intensity—an effect of doubtful therapeutic value in the circumstances. Eradication of the anopheline carriers (and anything less than eradication would probably be useless) is practically impossible. Bonification in such a district as this, is *par excellence*, inapplicable. There remains the unorthodox

attitude of non-interference and allowing the malaria to continue in its present mild (and yet excessively parasitised) course until, as apparently in some of the lower forms of life, a state of perfect commensalism is reached. There is no reason why further research into the nature of malarial immunity should not disclose a means of hastening and perfecting this end. In the meantime, a vigilant watch must always be kept for the incidence of those conditions, which, sometimes so appallingly, upset the balance in a hyperendemic community and cause a fulminant outbreak.

SUMMARY.

1. An investigation was carried out in a forest area in Kenya Colony (*a*) to determine the anti-malarial effect of atebirin and plasmoquine and (*b*) to study the course of hyperendemic malaria, both over a period of a year.

2. One gramme of atebirin per person monthly (less for children) in ten separate doses and 0.1 gramme of plasmoquine were given to the inhabitants of two villages respectively, each containing just under 50 people, and a third village was used as a control.

3. The absence of suitable indices for the measurement of morbidity in a hyperendemic area is pointed out; the following, *faute de mieux*, being used:— (*a*) Parasite rate; (*b*) Spleen rate; (*c*) Parasite intensity; (*d*) Fever rate.

4. The only index which was affected by the administration of the drugs was that of parasite intensity, which after 12 months was approximately halved in the treated villages and showed a rise in the control. It is pointed out that unlike epidemic or endemic regions this does not in a hyperendemic area necessarily represent an effect of any therapeutic value.

5. Hyperendemic malaria in Taveta is characterised by the mildness of the symptoms it gives rise to, in spite of heavy blood infection and in contrast to its deadliness to Europeans.

6. One third of the inhabitants showed no fever at all during the year and on an average an individual had only one attack during this period.

7. Of the 10 infants living in the experiment villages, 4 died during the course of the investigation and of those 4 deaths only 1 could be definitely ascribed to malaria alone. Benign-tertian appears to cause a greater mortality than that of the other species—unlike the position elsewhere.

8. A subsidiary investigation into 67 children of 12 months and under revealed the following points with regard to age incidence:—

(*a*) 100 per cent. malaria infection after 6 months.

(*b*) Sub-tertian and benign tertian malaria begin in the second and third months respectively, whilst quartan is delayed till the 5th month.

(*c*) Nearly 100 per cent. of the children of a year old are infected with quartan.

(d) There was no special monthly variation in the crescent rate, but approximately a half of the infants of all ages had crescents.

9. Incidence of malaria later in childhood and in maturity followed the recognised course of hyperendemicity, *viz.* round the 80 per cent. level in children, 30 per cent. in young adults and 25 per cent. in later life. Quartan malaria disappears much more rapidly than sub-tertian and gametocytes of the latter more rapidly than asexual forms.

10. The prevalent species is *Plasmodium falciparum*, with *P. malariae* a close second. *P. vivax* is comparatively uncommon and tends to have a focal distribution.

11. Seasonal changes in the parasite or fever rates were slight. New quartan infections appeared about three months after the rainy season and benign tertian a month. *P. vivax* and to a less degree *P. malariae* have a habit of appearing fleetingly for one month and not re-appearing until 8 or 9 months later. Crescent rates likewise show little seasonal changes.

12. The morphology of *P. falciparum* is similar to that described elsewhere by the writer as characteristic of an East African strain or sub-species. Certain peculiarities of *P. malariae* are described.

13. *Anopheles gambiae* is the principal transmitter of the disease and to a lesser extent *A. funestus*.

14. It is suggested that in this highly hyperendemic region a condition of commensalism is slowly being reached—as shown by the mildness of the malaria in contrast to the high degree of infection—and that because of the inapplicability of the ordinary measures of control, the attainment of this state should be the aim.

PROTOCOLS.

PROTOCOL 1 (MBOGONI 28). MALE CHILD AGED 6 MONTHS.

Heavy malaria infection (triple) and slight fever.

Date.	Spleen. Finger- breadths.	Fever.	Sub- tertian.	Crescent.	Benign Tertian.	Quartan.
4. 8.33	—		50	50	—	50
6.10.33			—	—	—	++
6.11.33			+	—	—	+
5.12.33		99.8 °F. 8.12.33	+	—	—	+
2. 1.34			+	+	—	+
14. 3.34			+	+	—	+
2. 5.34		100.2 °F. 10.5.34	+	+	—	+
11. 6.34			+	—	—	+
10. 7.34			+	—	—	+
16. 8.34		99.2 °F. 23.8.34	+	—	—	+
8. 9.34			40	+	—	50

PROTOCOL 2 (SELANDONGO 48). MALE CHILD AGED 1 YEAR.

Heavy malaria infection with absence of fever.

Date.	Spleen. Finger- breadths.	Fever.	Sub- tertian.	Crescent.	Benign Tertian.	Quartan.
2. 8.33	1		$\frac{40}{50}$	+	—	+
7.10.33			+	—	—	—
7.11.33			+	—	—	—
6.12.33			+	—	—	—
3. 1.34			+	—	—	—
15. 3.34			+	—	—	—
2. 5.34			+	+	+	—
12. 6.34			+++	+	—	—
11. 7.34			+	—	—	—
16. 8.34			+	+	—	—
8. 9.34	3		$\frac{40}{50}$	+	—	—

PROTOCOL 3 (MSENGONI 43A). MALE CHILD BORN 13.3.34.

First attack of malaria in an infant is unaccompanied by fever.

Date.	Spleen.	Fever.	Sub- tertian.	Crescent.	Benign Tertian.	Quartan.
15. 3.34	—		—	—	—	—
6. 4.34			—	—	—	—
2. 5.34			—	—	—	—
12. 6.34			+	+	—	—
11. 7.34	—		+	—	—	—
16. 8.34			+	+	—	—
7. 9.34			$\frac{30}{50}$	+	—	—

PROTOCOL 4 (MBOGONI 38). MALE ADULT AGED 30 YEARS.

Scanty parasites but several attacks of mild fever.

Date.	Spleen. Finger- breadths.	Fever.	Sub- tertian.	Crescent.	Benign Tertian.	Quartan.
1. 8.33	3		—	—	—	—
6.10.33			—	—	—	—
6.11.33			—	—	—	—
5.12.33			—	—	—	—
2. 1.34		99.4 °F. 20.2.34	+	—	—	—
14. 3.34	2		—	—	—	—
2. 5.34		99.8 °F. 4.5.34	—	—	—	+
11. 6.34		100.2 °F. 7.6.34	—	—	—	—
10. 7.34		99.0 °F. 27.7.34	—	—	—	—
16. 8.34			—	—	—	—
7. 9.34			—	—	—	—

PROTOCOL 5 (MSENGONI 22). MALE CHILD AGED 2 YEARS.

Possibly new infection of benign tertian in June and quartan in August.

Date.	Spleen. Finger- breadths.	Fever.	Sub- tertian.	Crescent.	Benign Tertian.	Quartan.
7.10.33	—	99.8 °F. 11.9.33	+	+	—	—
7.11.33			+	—	—	—
6.12.33		100.0 °F. 13.12.33	+	++	—	—
3. 1.34		99.2 °F. 20.1.34	+	+	—	—
15. 3.34		99.8 °F. 10.3.34	+	—	—	—
6. 4.34			+	+	—	—
2. 5.34			+	+	—	—
12. 6.34			—	—	++	—
11. 7.34		100.6 °F. 2.7.34	+	++	—	—
16. 8.34			+	—	—	++
7. 9.34	2		$\frac{40}{50}$	$\frac{10}{50}$	—	$\frac{30}{50}$

PROTOCOL 6 (MBOGONI 18). MALE CHILD AGED 4 YEARS.

Tendency of sub-tertian to disappear in presence of benign tertian parasites.

Date	Spleen. Finger- breadths.	Fever.	Sub- tertian.	Crescent.	Benign Tertian.	Quartan.
5. 9.33	4		+	—	—	—
6.10.33			+	—	+	—
6.11.33			+	—	—	—
5.12.33			—	—	—	—
2. 1.34			+	—	—	—
14. 3.34			+	—	+	—
2. 5.34			+	—	—	—
11. 6.34			—	—	++	—
10. 7.34		99.8 °F. 14.7.34	—	—	—	—
16. 8.34			+	—	—	—
7. 9.34	3		—	—	+	—

PROTOCOL 7 (SELANDONGO 52A). MALE CHILD AGED 1 MONTH.

Fatal case of benign tertian malaria. Plasmoquine fails to prevent infection.

Date.	Spleen.	Fever.	Sub- tertian.	Crescent.	Benign Tertian.	Quartan.
15. 3.34	—	—	—	—	—	—
6. 4.34	—	—	—	—	—	—
2. 5.34	—	—	—	—	—	—
12. 6.34	—	99.2 °F. 14.6.34	—	—	++	—

(Died 30.6.34 ? diarrhoea or ? malaria).

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A SPLEEN RATE SURVEY IN THE COLONY OF HONGKONG.

BY

K. H. UTTLEY, M.A., M.D., D.T.M. & H.

INTRODUCTION.

A large number of reports have been published in the past giving details of spleen rates in the East, but not many have been concerned with China.

MELENEY and LEE (1927) obtained a rate of 10·4 per cent. in some villages near Peking, and the National Health Administration of Nanking (1933) working in the Yangtse Valley of Central China examined 14,015 children, meeting with 436 (3·04 per cent.) palpable spleens. In the city of Nanking they obtained a spleen rate of 2·46 per cent. As far as I can ascertain, no reports have appeared in the literature dealing with spleen rates for South China, excepting certain ones to be mentioned later in this paper, which deal with Hongkong.

Before summarising the work done in Hongkong, it will be well to consider the relevant geographical features of the Colony, and the mosquitoes there concerned with the transmission of malaria.

Geographical Features.

The Colony of Hongkong lies just within the northern limits of the tropics, south of the mainland mass of China, near the estuary of the Canton or Pearl River, and facing the China Sea. It consists of :—

a. The Island of Hongkong, 32 square miles in area, on which is the city of Victoria, with an estimated population of 424,132.

b. The Peninsula of Kowloon, 2½ square miles in area, and an estimated population of 297,293.

c. The Leased or New Territories, of about 300 square miles in area, and an estimated population of 101,298. There is in addition, an estimated population of 100,000 people living on the junks and Chinese boats of the Colony. The New Territories are very hilly, the hills rising to an average height of 1,500 feet, and interspersed with numerous valleys and ravines. Except in the lower parts of the valleys, there are not many trees, and the general appearance of the countryside is one of barren and rugged hills, with narrow cultivated valleys in between them, except to the North and West, where there are several fairly wide valleys with flat areas two or three miles in extent, given over to the growing of rice.

The population is scattered about in small villages lying in the valleys and plains, nearly all of them engaged in agriculture, and having little or nothing to do with the cities of Victoria and Kowloon.

Very few villages are more than half a mile from the foothills, and although later on in the paper I shall refer to the spleen rate in the plains, this fact must be borne in mind.

Mosquito Carriers.

The chief mosquito carriers, as determined by the Government Malariologist (JACKSON, 1932) are *Anopheles minimus* and *A. jeyporiensis* var. *candidiensis*. JACKSON (1933) found *A. maculatus* and *A. hyrcanus* var. *sineusis* to be carriers under exceptional conditions at a labour camp, but their infection rates were low as compared with those of the first two species, derived from the same source.

Previous Spleen Rate Surveys in the Colony.

The following summarizes the surveys carried out by the Government Malariologist, and details may be found on reference to his various reports (JACKSON, 1931, 1932, 1933).

a. 391 children examined in the Eastern end of the city of Victoria had a spleen rate of 2.30 per cent. (JACKSON, 1931).

b. Of 65 children examined in Stanley, a native village on the Island of Hongkong, none had palpable spleens (JACKSON, 1931).

c. Several small surveys in the New Territories showed that of 93 children examined, 24 or 25.8 per cent. had palpable spleens (1931).

d. Of 400 children examined in different parts of the Colony 96, or 24.00 per cent. had enlarged spleens (1932).

e. Five surveys carried out, some on the Island and some on the Mainland during 1932 showed that of 304 children, 18, or 5.9 per cent. had palpable spleens (1933).

Altogether during the three years 1930-1932, 1,253 children were examined, and 147 or 11.7 per cent. had enlarged spleens. The above is all that was known about spleen rates among the children of the Colony prior to the survey about to be considered.

DETAILS OF THE SURVEY.

It was decided to carry out a spleen rate survey of as many of the village school children of the New Territories as possible.* This was done during the 20 months from March, 1933, to November, 1934. As hardly any children come to school before 5 years of age, or stay on after 14, the figures below refer to rates for children between those two ages.

*Each village school caters for the children in the village concerned. Hardly any children will go to school in a neighbouring village, consequently the spleen rate in the village school will correspond closely with that of all the children in the village.

For the purposes of this paper, I have divided up the Colony into three areas : i, Hill and foothill areas ; ii, Plains ; iii, Cheung Chow Island.

i. *Hill and Foothill Areas*.—Children examined : 2,609 ; spleens palpated : 272 = 10.8 per cent.

ii. *Plains*.—Children examined : 1,675 ; spleens palpated : 82 = 4.9 per cent.

iii. *Cheung Chow Island*.—Children examined : 375 ; spleens palpated : 4 = 1.1 per cent.

Total for all districts : Number of children examined : 4,659 ; spleens palpated : 338 = 7.47 per cent.

It will be seen from the above figures, that there is a tendency for the spleen rate to be lower in the plains than in the hill and foothill areas. This is not more than a general tendency, for low spleen rates were sometimes found in villages at the foothills, though hardly ever was a high spleen rate found away from the hills.

The case of Cheung Chow Island is of interest. It is a small island of a few hundred acres in extent, situated half a mile from the nearest land. There is little water on the island, a considerable quantity of what is used has to be brought over in boats. Although some anophelines have been caught there, there are not many suitable breeding places.

I endeavoured to find out if there was any difference between the spleen rate values in summer and in winter, having in mind a paper of LYENGAR and SUR (1929) in which they state that in parts of India there is a definite seasonal variation. In this survey the difference was so small as to come within the limits of experimental error.

There seemed to be little uniformity as regards the rate in apparently similar geographical areas. The rate varied from 1.4 per cent. in Sai Kung, a village situated at foothills near the sea, to 41.4 per cent. in the Castle Peak area, in a similar situation.

Below the results are grouped in districts, the first four of which are in the plains.

District.	Children Examined.	Palpable Spleens.	Percentage.	District.	Children Examined.	Palpable Spleens.	Percentage.
Fan Ling	637	29	4.6	Tsuen Wan	280	15	5.4
Yuen Long	631	28	4.4	Sai Kung Village	71	1	1.4
Ping Shan	314	29	9.2	Peninsula East			
Kam Tin	276	20	7.2	and South of			
Tai Po	1,124	71	6.3	Sai Kung	147	51	37.7
Sha Tin	205	23	11.2	The Islands	536	15	2.8
Sha Tau Kok	310	23	7.8				
Castle Peak	128	53	41.4	Total	4,659	338	7.47

No work has yet been done to ascertain the blood parasite rates in these localities, so that it is not possible to say definitely whether or not one area is more highly infected with malaria than is another, but excepting for the Island of Cheung Chow above discussed, the impression obtained by the medical officers is that there is much less malaria in the plains of Yuen Long and Fan Ling, than in the foothills and hills. In the latter areas, considerable numbers of malaria cases are met with, and the inhabitants are well acquainted with the disease, giving typical histories in many cases, even in those places with low spleen rates.

During the latter half of the survey, note was taken of the sizes of the spleens encountered. The following are the details of size and number of the 204 spleens measured.

Finger Breadths.	Number.	Percentage.	Finger Breadths.	Number.	Percentage.
1	29	14.4	6	12	5.9
2	43	21.1	7	11	5.4
3	45	22.1	8	8	3.9
4	40	19.6	9	—	—
5	14	6.8	10	2	0.6

The above figures are not large, but they are of interest because of the dearth of such details concerning China, and South China in particular.

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ATEBRIN, PLASMOQUINE AND QUININE IN THE TREATMENT OF MALARIA.*

BY

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I.—MODERN VIEWS ON IMMUNITY AND ITS BEARING ON TREATMENT.

In the *Report of the Malaria Commission of the League of Nations* (1933), in the course of an exhaustive survey of the literature dealing with malarial therapeutics which had been published up to that time, repeated reference is made to the fact that malaria is a disease which varies widely in its virulence, not only in different countries but according to the strain of parasite which is responsible for the infection concerned. In Kenya it is noticeable that pernicious types of sub-tertian malaria and cases of blackwater fever occur much more frequently in some districts than in others, a fact that offers an explanation for the wide divergency of the views held by equally competent and experienced medical men as to the need or otherwise for injection treatment. In the vicinity of Kisumu on Lake Victoria it is found to be impossible to treat more than a minority of cases with quinine alone. To quote from the League of Nations Report " . . . to existing knowledge that some species of the malarial parasite are much more virulent to the host than others has been added the information that there is a difference between the clinical virulence of the different geographical races or strains of the same morphological species," and again (1933), " it has been found, for example, that to control the primary attack of cases of malignant tertian malaria infected with a Rome strain of *P. falciparum* it required eight times as much quinine as was required to control cases infected under precisely the same conditions with an Indian strain. . . . These and other results afford a reasonable explanation of contradictory observations on the therapeutic efficacy of the same plan of treatment conducted in different countries and indicate the importance of local therapeutic investigations."

In view of this last recommendation, an attempt has been made to carry out a clinical experiment to determine the relative values of certain anti-malarial

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remedies in the treatment of local strains of malaria and to ascertain whether the higher cost of such drugs as atebrin and plasmoquine is so far offset by their greater efficacy as to justify their routine use in native hospitals.

The type of malaria which predominates heavily in this Colony is malignant tertian and all the cases included in the experiment were infected with that species of parasite. With few exceptions, the patients were adult males and were drawn from a variety of tribes subject to malarial infection in widely separated parts of the Colony. It was considered impossible even by careful interrogation to arrive at an accurate decision as to the nature of the attacks, whether primary infection, relapse, or secondary infection; and even had it been possible to follow up cases with a view to estimating the relapse rate, malaria was so rife throughout the period that the figures would have been rendered worthless by the impossibility of determining whether further attacks were in fact relapses or were due to re-infection.

The view is being put forward with increasing emphasis that it is bad practice to begin the treatment of malaria infections at the commencement of the attack since by so doing one prevents the patient from developing an acquired immunity. In this connection the League of Nations Report has a good deal to say, though it recognises the danger that may attend delay in treating malignant tertian cases. We read as follows (1933), "... to give large doses of quinine indiscriminately to all cases of malaria is a grave error ... we wish to correct the common view that the earlier one begins specific treatment the more successful will be the results," and lower down on the same page, "... the risk of pernicious symptoms in malignant tertian fever is greatest in the primary attack and earlier recrudescences, because any person who has recovered from that stage of the disease has acquired sufficient defensive power to protect him when he gets a relapse, against those disastrous consequences."

Though this dictum may be true enough in theory and though it is an undoubted fact that many people do develop an acquired immunity to malaria, it is a common experience to find in East Africa that pernicious symptoms do from time to time arise in old residents who have successfully weathered many attacks of malaria, a fact which cannot always be explained by the possibility that they have been infected anew with a strain of parasite against which they have no immunity. Further, there is the danger that such conditions as malarial cachexia and blackwater fever may result if vigorous treatment is not commenced early, so that if one waits for a patient to develop immunity one may run a grave risk of his losing his life in the process. The impracticability of the suggestion embodied under this head in the *Report of the Malaria Commission of the League of Nations* was pointed out by DUBOIS (1933) who is strongly of the opinion that treatment in sub-tertian malaria should be commenced at the earliest possible moment.

J. GORDON THOMSON (1933) states that acquired immunity may be highly dangerous to Europeans who still harbour parasites in their blood, are usually

anaemic and run the risk of contracting haemoglobinuric fever. He further states that acquired immunity in natives results from their being bitten constantly by infected mosquitoes over a period of from twelve to fifteen years and those who have worked in hyperendemic areas may well consider that the price paid in infant and child mortality for this dearly-won freedom from clinical manifestations is too heavy. In any case, it is doubtful whether the degree of immunity acquired by Europeans resident in East Africa is, in view of its attendant risks, worth having, while in questioning the desirability of treating native children for their malaria, the League of Nations Commission may be advocating the survival of a few immunes at the expense of a large number of prematurely-dead infants.

That the introduction of malaria-therapy into asylum practice has opened up a very wide field for research, cannot be questioned, but it is questionable whether so many sweeping conclusions could have been drawn and widely accepted without challenge in any other branch of biological research, if it had been published beforehand that all the experimental animals used were diseased. The mere fact that there is a close relation between para-syphilis and malaria is surely enough to give pause to the assumption that clinical experiments in this field have any but a very limited application in malariology generally. GILL (1933) draws attention to these possible fallacies when he says that not only are the subjects of these oft-quoted tests syphilitics, but they are patients who have been for years under treatment with various arsenical and other remedies which may have profoundly altered their reaction to malarial infection, anti-malarial treatment and the development or otherwise of an acquired immunity. He epitomises his remarks by stating that these cannot be regarded as "clean experiments."

The fact that in some districts the whole native population is subject to malarial infection from earliest youth, cannot be denied and to quote again from the League of Nations Report (1933), "It is known . . . that in some localities of Africa and other tropical countries, the entire native population above one year of age harbour malarial parasites in their blood continuously, although no one above a few years of age suffers any appreciable illness attributable to the parasitic infection." The first half of this statement has been admitted to be true, but the existence of the implied complete immunity arising out of this state of affairs is open to question. McLEAN (1931) working in South Kavirondo, Kenya, records the fact that of 467 natives from whom blood smears were examined for trypanosomiasis 451, or 96.57 per cent., were found to be harbouring malaria parasites. The gametocyte rate was 9.8 per cent., and it is stated that the people were not apparently inconvenienced by their infection. In another area in Central Kavirondo the parasite rate was 95.1 per cent. of 203 children under twelve and the gametocyte rate was 5.9 per cent. These are recorded facts which cannot be called into question but the conclusions drawn from them are less well proven. The advent of some factor which

porarily or permanently lowers the resistance of these parasitised natives well enable the plasmodium of malaria to turn the tables against this vaunted immunity. It is a common experience for patients to be admitted to hospital on some surgical or medical condition other than malaria and to require treatment for that disease as well as for that for which they first sought advice. Working in the same hyperendemic area near Lake Victoria as that referred to by McLEAN the writer saw a good deal of illness caused by malaria, and over a period of two years in that district treated three cases of blackwater fever, and one other, all in local natives, two of whom died.

It is also stated in the Report that crescents are seen much more frequently in primary attacks since the establishment of an acquired immunity discourages their development, but in the light of the clinical evidence to be dealt with shortly, these statements are difficult to correlate with two points of importance. Firstly the incidence of crescents in all the groups of patients was exceedingly high, and secondly, a large proportion of the patients must have had a good deal of malaria previously. The number of patients found to have crescents was 12 per cent. and this high figure cannot be attributed to any supposed provocative action of either atebrian or plasmoquine since even in those cases treated with quinine alone, the gametocyte rate was 50 per cent. It may be that although a number of the patients concerned came from true endemic areas, they may have been previously infected with local strains of malaria, but it is unlikely that many of the total of 226 subjects had escaped several attacks while residing in the artificial endemic area of Nairobi township.

II.—CLINICAL INVESTIGATION INTO TREATMENT.

The primary object of the investigation was to ascertain the relative merits of atebrian and quinine in the treatment of local strains of malaria and to determine possible whether the alleged higher standard of efficacy of the former would so offset its higher cost as to warrant its routine use in Government Native hospitals. Since the actual cost of atebrian, dose for dose, is so much higher than that of quinine, the only ways in which a saving could be effected were by reducing the time of stay in hospital or by preventing relapses.

It should be noted that so far as these records are concerned, it was the rule for patients to give a history of having been ill for some days before admission to hospital so that one desideratum laid down by the League Commission was met perforce. Be that as it may, it is, of course, quite out of the question when a patient is admitted to hospital to do anything else but restore him to health by vigorous treatment at the earliest possible moment for the following reasons. Firstly because the fear of the development of pernicious symptoms must come largely in the doctor's consciousness, even if such symptoms are not already present on admission; secondly, the pressure on beds when malaria is rife makes it imperative for obvious reasons of humanity to discharge every

patient at the earliest safe moment; thirdly, there is the ever-present question of the expense to whatever party, usually Government, has to bear the cost of maintaining the patient in hospital; and fourthly, the employer of labour wants his servant back earning his wages as soon as he is fit to do so. However ill-advised and unfortunate any or all of these factors may be, not one of them can be ignored and they combine to demand that the beginning of treatment shall coincide with admission to hospital.

Though it is still impossible to say how quinine acts as a curative agent in malaria, some workers, leaning towards the view that it functions as a sort of biological catalyst, assert that small doses are just as efficacious as larger ones and that since they are less likely to give rise to undesirable symptoms they may even be preferable. While it is perfectly true that in the past, quinine was often pushed beyond the true limit of tolerance, a practice which can hardly be defended by any serious worker to-day, it would appear to be dangerous to make a general statement on this point. Different subjects vary widely in their tolerance of quinine and each case, particularly where women are concerned, requires to be treated on its own particular merits. Where robust males are concerned, however, it is the writer's opinion, backed by the clinical evidence to be cited later, that a dosage of less than 30 grains of quinine daily, is inadequate for the treatment of sub-tertian malaria.

General Remarks upon Atebrin Alone and with Plasmoquine.

In reading the extensive literature which has been built up upon the subject of atebrin treatment, it is at once apparent that the earlier hopes that it could almost always prevent relapses in sub-tertian malaria, have not been realised. Reports are conflicting but the general consensus of opinion seems to be that while relapses do occur they are far less frequent after atebrin than is the case after quinine, a view which is upheld by the writer's own experience in treating European cases in Nairobi. As was noted above, it would have been impossible so far as the native cases in this experiment were concerned either to have kept the patients under observation over a period of months or to have eliminated the possibility of their becoming re-infected.

In treating Europeans it is found after but a little experience that atebrin exerts its therapeutic action more slowly than does quinine but it has never been necessary to treat a case for more than 7 consecutive days with one tablet of 0.1 gramme three times daily, and whereas patients on full doses of quinine are very often made uncomfortable by that drug, in the sense that they have the usual manifestations of cinchonism, there are no parallel discomforts to be recorded in connection with atebrin. The result of this has been that when a course of atebrin has been completed the return to normal health has been more rapid and moreover the treatment is over and done with and not prolonged over an indefinite period of weeks and months, as is still done in a large number

of cases with quinine. The yellow staining of the skin, is the only unusual phenomenon noted and since it is non-toxic it can be ignored. When atebrin and plasmoquine are given in combination as was originally recommended, a percentage of cases occur in which abdominal pain is a prominent symptom, so prominent as sometimes to dominate the clinical picture. There is abundant evidence to show that plasmoquine exerts little or no effect upon the asexual stages of the sub-tertian parasite, and since it is presumably able to exert none but its own specific therapeutic action whether given alone or in combination with other drugs, there would appear to be no justification further to upset the patient during a serious illness by giving him this pain. There appears to be no valid reason why the plasmoquine should not be administered as a separate course after the attack of malaria has been terminated by treatment, since by that means the desired end of crescent-elimination can be gained without any discomfort. The League of Nations Report (1933) says, "Clinicians should try to throw off the belief that by one or other form of intensive drug treatment during the primary attack they can sterilise all the parasites in infected persons," but adds this rider, "the justification for aiming at early permanent cure by specific drug therapy is much greater in malignant tertian than in benign tertian malaria." The Commissioners might have gone further than that and laid down complete cure as the only sane object to have in view during the treatment of any case of sub-tertian. Whatever view one may hold on this point or on the ability or otherwise of plasmoquine to prevent relapses, it can hardly be questioned that to reduce enormously the number of people having gametocytes in their peripheral blood can hardly fail to be beneficial to the public health and it is just this which plasmoquine is able to do.

For reasons already given it was not possible to keep the subjects of this experiment in hospital for longer than was necessary and since to have given atebrin and plasmoquine separately would have involved doubling that time, it was decided to combine the courses. It was found that the tendency to develop abdominal pain was not present in native patients and in the whole series of 226 cases no toxic symptoms of any kind arose.

In the tables which follow, groups are shown separately in which atebrin was combined firstly with plasmoquine simplex and secondly with plasmoquine compound. This was done not because of any supposed superiority of one drug over the other but because supplies of the latter were available for use. Normally plasmoquine simplex would have been used and would be given over a period of 5 days in a dosage of 0.01 gramme three times daily.

Methods of Treatment Employed.

In order to make the fairest possible comparison between atebrin and quinine therapy, the following procedure was adopted. All cases diagnosed before admission as being clinically malaria, were given calomel and a saline purge and on the receipt of a positive blood report were labelled with a number.

The numbering being consecutive, the cases fell haphazard into their places whether they happened to be severe or slight; and then odd numbers were treated with the drug or drugs under test, while the even numbers were treated with quinine and regarded as controls.

The examination of slides was carried out as far as was possible by the same person and a negative finding was recorded only after a thorough search had been made of both thin and thick films.

In a number of cases, the patients remained in hospital for periods longer than the tables cover, but though for reasons of economising space particulars of the later days are not included, the details were used in assessing final results. and in calculating averages.

The following are the doses of drugs given in each case three times daily : atebirin 0.1 gramme ; plasmoquine simplex 0.01 gramme ; and plasmoquine compound, 0.01 gramme plasmoquine with 0.125 gramme quinine. These doses represent, of course, the standard tablets as sold by the manufacturers. The dose of quinine for the controls was 10 grains in solution three times daily, *i.e.*, 0.6 gramme.

The number of cases treated with (1) atebirin alone was 35, and the corresponding quinine controls 35 ; while the number of cases treated with (2) atebirin and plasmoquine compound was 31, and the corresponding quinine controls 31. The cases treated with (3) atebirin and plasmoquine simplex were 22, and the quinine controls 22 ; while the cases treated with (4) quinine 15 grains daily were 25, and the quinine controls 25.

From the protocols the following four bases of comparison may be extracted and it is proposed to summarise the results under those heads.

I. Length of time in hospital. (Calculated from the day when treatment was commenced.)

II. Persistence of fever.

III. Persistence of asexual parasites.

IV. Persistence of crescents.

I.—LENGTH OF TIME IN HOSPITAL.

	Test Drug.	Days.	Quinine Controls. Days.
1	Atebrin	6.8	7.9
2	Atebrin + plasmoquine co.	7.1	7.9
3	Atebrin + plasmoquine simplex	7.7	8.8
4	Quinine grains xv daily	9.1	7.6

This analysis establishes at once the fact that patients receiving atebtrin alone or in combination, were able to leave hospital roughly a day sooner than those who were treated with quinine. That patients on atebtrin seem to revert more rapidly to normal health than those on quinine is just as noticeable in African practice as it is when Europeans are being dealt with. It is noticeable that the addition of plasmoquine although it does not give rise to toxic symptoms, does retard the return of a normal sense of well-being. It will be seen that the patients who received only 15 grains of quinine daily were longer in hospital than those in any other group.

The combined averages are as follows :—

1. Atebrin alone or in combination, 7·2 days.
2. Quinine 30 grains daily, 8·05 days.
3. Quinine 15 grains daily, 9·1 days.

II.—PERSISTENCE OF FEVER.

	Test Drugs.	Days.	Quinine Controls. Days.
1	Atebrin	2·9	2·8
2	Atebrin + plasmoquine co.	3·0	2·6
3	Atebrin + plasmoquine simplex	2·5	2·9
4	Quinine grains xv daily	3·3	2·6

These figures tend to confirm the view expressed above and constantly referred to in the literature, that atebtrin does not terminate the fever of a malarial attack quite as rapidly as does quinine in full doses, but a study of a large number of temperature charts shows that whereas quinine ends the attack by a process resembling lysis, atebtrin, when once it brings the temperature down, more often prevents it rising again.

Again in this analysis, the cases treated with small doses of quinine give the least favourable figures. The combined averages are :—

1. Atebrin alone or in combination, 2·8 days.
2. Quinine 30 grains daily, 2·7 days.
3. Quinine 15 grains daily, 3·3 days.

III.—PERSISTENCE OF ASEQUAL PARASITES.

	Test Drug.	Days.	Quinine Controls.	Days.
1	Atebrin	3.0	3.6	
2	Atebrin + plasmoquine co.	2.5	2.6	
3	Atebrin + plasmoquine simplex	2.5	3.0	
4	Quinine grains xv daily	2.6	3.0	

The persistence of asexual parasites in the peripheral blood is not of itself a matter of very great moment, but in spite of numerous references to the subject in scientific literature, there still persists in the minds of many practitioners a belief that if a patient has taken a single dose of quinine, it is futile to take a blood slide for diagnostic purposes. Not only does this result in a lazy habit of treating all minor febrile attacks as being due to malaria but it also deprives the doctor of definite information as to the species of parasite causing the illness. In this analysis the patients treated with small doses of quinine give the best results.

The combined averages are :—

1. Atebrin alone and in combination, 3.0 days.
2. Quinine 30 grains daily, 3.0 days.
3. Quinine 15 grains daily, 2.6 days.

IV.—PERSISTENCE OF CRESCENTS.

	Test Drug.	Percentage of cases in which crescents persisted.	Quinine Controls.
1	Atebrin	80	59
2	Atebrin + plasmoquine co.	7	68
3	Atebrin + plasmoquine simplex	18	86
4	Quinine grains xv daily	68	76

It is not suggested under this head that because crescents had disappeared from the finger-blood at the time of discharge from hospital, the patient had been permanently freed from gametocytes. It is recognised that in some patients in whom no crescents were found at any stage, they may have developed sub-

sequently, or alternatively, that their disappearance, where they did disappear, was purely temporary. It is suggested, however, that the very much lower figures obtained for patients who received plasmoquine indicate that it is very much more likely that the crescents were destroyed altogether in these cases than it was in those treated with atebtrin or quinine alone. The averages were : for atebtrin cases 80 per cent. persistence of crescents, for cases receiving plasmoquine, $12\frac{1}{2}$ per cent., and for cases on quinine 71 per cent.

ATEBRIN IN BLACKWATER FEVER.

Whenever in the course of a case of blackwater fever it is found that malaria parasites are still present in the blood, it is difficult to avoid the feeling that one is in a quandary. The administration of full doses of quinine may precipitate a further haemolysis and the alternative of working up from very small doses involves considerable delay. In addition to declared cases of blackwater fever, there are the subjects who present themselves for treatment while they are still in the condition described by MANSON-BAHR as the "pre-blackwater state," whose position is so precarious that a single dose of quinine may precipitate a haemolysis. Both established cases of blackwater and patients obviously in danger of becoming subjects of that disease, appear to tolerate atebtrin in full doses without the risk of primary or secondary haemolysis. Two Africans and one Indian, whose blackwater fever was complicated by persistent subtertian malaria infection, were successfully treated with atebtrin. The usual measures were of course adopted for the treatment of the more serious disease, and all three recovered without relapse on 0.1 gramme of atebtrin given three times daily for 5 days. The writer has had the opportunity of treating two Europeans whose appearance and general condition led to the belief that their heavy sub-tertian infections were likely to culminate in severe haemolysis. In both cases atebtrin 0.1 gramme was given 4 hourly until the temperature fell to normal and both recovered without the development of any complication.

COST OF TREATMENT.

With regard to the treatment of Europeans it may be said at once that the cost of a single day in hospital more than balances the added cost of atebtrin over that of quinine. Besides this there is the consideration that convalescence is both quicker and more complete. The same remarks apply in only a slightly lesser degree to Asiatics but where Africans are concerned, the position is not quite so clear.

Assuming that while he stays in hospital every African malaria patient takes 30 grains of quinine a day, the cost of drugs and feeding works out as follows :—

A. <i>Quinine</i> —					Shillings.
Quinine grains	30	daily	for an average of	8.3	days
at	2	shillings	per ounce	1.03
Cost of food	at 0.34	shillings	per day	for 8.3	days .. 2.82
TOTAL					3.85
B. <i>Atebrin</i> —					
Cost of 15 tablets of atebrin	at 13s.	1½d.	per 100		
tablets					1.97
Cost of food	at 0.34	shillings	per day	for 6 to 8	days.. 2.31
TOTAL					4.28

The cost of plasmoquine simplex is 22 shillings per 500 tablets or 0.66 shilling per patient receiving 15 tablets, but this drug is needed as much for cases treated with quinine as it is for those on atebrin so that for the purposes of this comparison the cost cancels out.

Thus the actual cost of treating a patient works out at 0.43 of a shilling more with atebrin than with quinine but it must be borne in mind that with the more expensive drug a whole day is saved, enabling 14 per cent. more cases to be treated with a given number of beds and also saving a definite amount of loss of labour to the employer.

SUMMARY.

Various aspects of immunity in malaria are discussed with particular reference to the *Report of the Malaria Commission of the League of Nations*. The view that there exist strains of malaria parasite which vary in their virulence is endorsed; but the principle that it is better not to begin treatment in sub-tertian malaria at once but to defer it, in order to encourage the development of an acquired immunity, is challenged on the ground that it is dangerous and impracticable.

It is considered to be doubtful whether Europeans ever develop a useful immunity to sub-tertian malaria.

The validity of conclusions drawn from clinical tests made in applying malaria therapy to para-syphilitics is questioned on the ground that these subjects are pathological.

Evidence is put forward in support of the view that small doses of quinine (15 grains a day) are less efficacious than moderate ones (30 grains a day).

Parallel series of cases were treated with atebrin and quinine. In some of the atebrin cases plasmoquine simplex or plasmoquine compound was given as well.

The length of time in hospital was a day less on atebrin than on quinine, but the time for the temperature to return to normal was slightly longer. There was not much difference in the rates at which parasites were eliminated from the peripheral blood by the two drugs.

The incidence of crescents in all groups of cases was unusually high but in those cases in which plasmoquine was given they disappeared from the peripheral blood in $87\frac{1}{2}$ per cent. as against figures of 20 per cent. with atebrin and 29 per cent. with quinine.

Atebrin was used successfully in three cases of blackwater fever complicated by sub-tertian malaria; and in several cases where a haemolysis threatened, without that event occurring after the exhibition of the drug.

Atebrin is cheaper in European practice than is quinine, and although in treating Africans in hospital it appears to be less economical to use atebrin, it would almost certainly prove to be cheaper in the long run and in any case its use would enable a given number of beds to be utilised by 14 per cent. more patients.

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ON THE FACTORS THAT MAY DETERMINE THE INFECTIVITY OF A TRYPANOSOME TO TSETSE.

BY

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In a paper in these TRANSACTIONS, CORSON (1935) records a remarkable instance of the infectivity of a trypanosome to tsetse.

A reedbuck was infected cyclically with a strain of *Trypanosoma rhodesiense*: some 3 weeks later a batch of about 120 clean *Glossina morsitans* was fed on the animal on two occasions, on the second of which 30 more clean flies were added. Of the 85 survivors dissected, 60 per cent. had infection of the salivary glands. A month later a second similar test was performed and of the 84 survivors 33 per cent. had infected glands.

As a control to this last test a monkey was infected with the same strain of *T. rhodesiense*. The day after trypanosomes were first seen in the peripheral blood of this monkey a batch of clean flies was fed on it, the opportunity of feeding being repeated on the two succeeding days. Dissection of the 90 survivors revealed only one fly with an infected gland.

It should be noted that the percentage of infected flies is in each case calculated from the number of flies that survive until the end of the experiment, and not from the total originally fed on the animal.

In his conclusions CORSON inclines to the view that "the special suitability of the reedbuck's blood rather than a selective change in the trypanosomes" affords the best explanation of the phenomenal infectivity of the trypanosomes to fly.

It is not clear whether the individual or the species reedbuck is intended. Presumably, too, "selective change in the trypanosome" means a change in the transmissibility of the strain as a result of residence in the antelope.

I feel that CORSON's paper calls for comment on the following two counts. First, a consideration of work which has already been done not only with the

reedbuck but with other species of antelope may throw further light on the significance of his observations. Secondly, in arriving at his conclusions CORSON has apparently overlooked the possible significance of ROBERTSON'S work on the endogenous cycle of a trypanosome in the vertebrate host. In making these criticisms I do not wish in any way to disparage CORSON'S remarkable achievement in demonstrating such a very high rate of infectivity.

Evidence was obtained years ago in Uganda (DUKE, 1912) which suggested that residence in antelope (bushbuck and reedbuck) resulted in an increased infectivity of a strain to tsetse. This enhancement of infectivity to tsetse was observed on two occasions; and in each case the quality was demonstrated in trypanosomes obtained in monkeys that had been inoculated with an antelope's blood, many months after its original infection. On the other hand recent work at Entebbe has shown that even in an antelope prolonged residence in one and the same host tends to weaken, suppress or even destroy altogether the power of a strain to infect fly.

We now know that several species of antelope suffer no apparent inconvenience when infected with *T. rhodesiense*, whereas other species, such as the Uganda forest duiker and the dik-dik, die rapidly.

At the commencement of an infection with *T. rhodesiense* in antelope, it is usual to find that the trypanosome is readily infective to tsetse. Reedbuck and bushbuck appear to be approximately equal in this respect; the oribi is perhaps somewhat less suitable to this trypanosome, but the evidence is as yet very limited. Thus the blood of a young adult reedbuck $3\frac{1}{2}$ months after its infection with a Tinde strain of *T. rhodesiense* was readily infective to tsetse (8.2 per cent. infected *G. morsitans* in 472 dissected DUKE, 1933). It is, however, significant to note how the percentage of infected flies varied on different days of the period during which the animal was under the test. The percentages were 10, 10, 3, 5 and 20, these figures being calculated from the total number of flies dissected after the first week or so in each experiment. This reedbuck remained in perfect health for many months, during which time the infectivity of its trypanosomes to fly diminished, until some 10 months after the first test 454 flies were fed on the animal without a single one becoming infected (DUKE, 1935). During the same period all inoculations of the animal's blood into monkeys were negative—altogether seven, of 10 c.c. each, between the 14th and the 26th months after the animal's original infection.

Now in any discussion on the infectivity of trypanosomes to tsetse it will I think be admitted that one cannot afford altogether to ignore the possibility of the occurrence in the vertebrate of a cycle of development, the phases of which are definitely related to the behaviour of the trypanosome in the intermediate host. ROBERTSON (1912) first called attention to this possibility. The essence of her view is that the trypanosomes in the blood of the vertebrate host vary from day to day in their infectivity to tsetse. Although the existence of this endogenous cycle is perhaps not yet proved—it is very difficult to obtain

absolute proof on this subject—the evidence that has accumulated since ROBERTSON'S original work in Uganda is on the whole confirmatory.

Let us consider CORSON'S results in the light of this view. First, the monkey experiment cannot be regarded a real control. For one thing there is some doubt whether a trypanosome in any given host attains its full power of developing in tsetse until a day or two after its first appearance in the peripheral blood. Secondly, if the three days when the flies fed on the monkey happened to coincide with a "negative period" (*sensu* ROBERTSON) of the cycle of the trypanosome in the monkey, this accident would completely upset any comparison.

A better control to the reedbuck results would seem to be the passages of the strain by fly from dik-dik, in which the percentage of infected flies varied between 5 and 10. But here the dates in the schedule for the dik-dik passages (CORSON, 1935) seem to show that the clean flies were put on each dik-dik not right at the commencement of each animal's infection (as was done with the first test on the reedbuck) but several weeks later, by which time the transmissibility of the trypanosome in the dik-dik might well have diminished from its original intensity. It will be seen that the infectivity percentage in the second test on the reedbuck is considerably less than in that of the first.

In a paper now in the press I submit figures which show the striking differences between the infectivity to fly of a trypanosome in one and the same animal from day to day, evidence which seems to lend support to ROBERTSON'S views.

It is highly probable, from the ease with which trypanosomes are transmitted at Tinde, that the conditions there are on the whole more favourable to transmission than those obtaining at Entebbe. Still more so in Nigeria, where the cycle in the fly is shorter and the infectivity rate at times very high. Whether these differences are due to fly, climate or trypanosome or to a combination of these factors cannot yet be determined.

LLOYD in Nigeria (1930), using the incubator, obtained in a single experiment 86.6 per cent. infected flies: a repetition of the experiment under apparently identical physical conditions resulted in only 8 per cent. infected flies. CORSON himself some years ago obtained a gland infection rate of 35 per cent. in a small batch of *G. pallidipes*. With such high potential infectivity the different phases of the endogenous cycle may produce relatively large differences. It is known that the balance may at times swing down to zero in a strain of relatively high infectivity.

If we hold that the African big game are the true and original hosts of the trypanosomes of the *brucei* group in that Continent, it is to be expected that among the antelope will be found species that are admirably adapted to the requirements of these parasites. Investigation so far has confirmed this expectation. On the other hand, typical *T. gambiense*, dependent on and consequently adapted to man for long ages, appears on the closer scrutiny devoted

to this subject in recent years to be less able to accommodate itself to antelope than *T. rhodesiense* and *T. brucei*. Doubtless much depends on the characters of individual strains. CORSON concludes that the special suitability of the reedbuck's blood affords the best explanation of his phenomenal figures. I believe he is right inasmuch as the blood of antelope affords optimal conditions to the trypanosomes of this species. But that the reedbuck is peculiar in this respect, I do not believe. In other species of tolerant antelope, *i.e.*, species which do not suffer any apparent inconvenience from the trypanosome, the same phenomenon will, in all probability, be found to occur.

As to whether the trypanosome has undergone a selective change, I believe that in a tolerant species of antelope *T. rhodesiense* will manifest its maximum transmissibility: restore it to a sensitive host, *i.e.*, one that succumbs rapidly to the trypanosome, and the transmissibility will diminish either at once or after a few passages in that species of animal. But even in the antelope, after a time, transmissibility will, I believe, diminish. This is at all events clearly indicated by my own experiments. It will therefore be of interest to learn whether the animal mentioned in this paper maintains anything like the high infectivity suggested by the two tests we have been considering.

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A NOTE ON THE BEHAVIOUR OF BABOON AND MONITOR BLOOD IN TSETSE FLIES.

BY

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Some 15 years ago I noticed in the intestinal contents of *Glossina palpalis* that had fed on the local species of baboon, *Papio tessellatus*, large masses of light green coloured crystals. From time to time since then the employment of baboons as food animals for the fly has served as a reminder of this phenomenon, which invariably follows the ingestion by the fly of baboon's blood. The crystals persist for some days after the meal on the baboon. They are found especially in the light brown and green portions of the digested blood, after the corpuscles have been disintegrated. They may be detected after several intervening meals on monkey or fowl blood, neither of itself contributes to their formation. In such circumstances they are plainly surviving from the last meal of baboon blood, and will eventually disappear if the fly abstains from this diet. No other mammalian blood hitherto fed to the tsetse has been found to yield large masses of these crystals. Guineapigs, rats, antelope, cattle, sheep, goats, dogs, fowls and monkey (a mangabey and three other species) have all at one time or another served as food supply to the tsetse at this laboratory, but in no instance have these crystals been found.

In digested human blood a few crystals sometimes occur. In flies fed on the monitor lizard (*Varanus*) crystals similar to some of the forms seen in digesting baboon blood are regularly present, but no record has been kept from which a detailed description can now be made.

The main types are two in number: long cylindrical crystals shaped like a whetstone or a needle, and a shorter form, in outline apparently like the text book illustrations of Charcot-Leyden crystals, but round or oval in cross section.

The long form may be blunt at the thicker end, tapering sometimes to a

point at the other extremity. Sometimes the thicker end carries a knob, or in the absence of the knob there may be a short peg projecting from the thick end of the crystal, like one end of a rolling pin. Sometimes the crystal tapers at both ends, one of which may carry a knob. The other shorter form varies greatly in size. It is this latter form which is found in digesting human blood. As already stated, every *Glossina palpalis* that has fed on baboon blood shows enormous numbers of these crystals in its intestine. They are also of regular occurrence in flies fed on *Varanus* blood and, if memory serves me, on crocodile blood also.

In baboon blood the length varies from 20 to 200 μ . The crystals also occur in other blood-sucking insects that have fed on baboon's blood, having been seen, in the second form chiefly, in culicine mosquitoes, tabanids, *Stomoxys* and the bed bug. In all these insects digestion is slower than in tsetse, and the crystals, associated as they are with the later stages of digestion, take 72 hours or longer to appear.

They occur also regularly and in enormous quantities in *G. morsitans* after a feed on the baboon; probably therefore in all species of tsetse in similar circumstances.

Now the baboon, as is well known, is immune against infection by natural means with the polymorphic trypanosomes. LAVIER (1928), of the League of Nations Sleeping Sickness Commission, attempted by various means to break down its resistance, but all to no avail. Different methods of introducing the parasites have been tried; heavy subcutaneous and intraperitoneal injections and exposure to a number of cyclically infected tsetse; similarly with *Varanus*. In Uganda all our attempts at infection of these two animals have failed, though REGENDANZ (1932) in Hamburg has succeeded in establishing a cerebro-spinal infection in the baboon without thereby infecting the blood stream.

Both the baboon and the monitor are accessible to *G. palpalis* on Lake Victoria, the latter animal and the crocodile being a very important natural food supply of this fly. It was therefore thought expedient to determine whether the ingestion of baboon or *Varanus* blood had any inimical effect on the development within the tsetse of the polymorphic trypanosomes. Experiments carried out with this object gave no very definite results, although they did demonstrate clearly that the polymorphic trypanosomes, *T. gambiense* and its allies, could develop fully in flies whose sole nourishment for two or three weeks was either baboon or *Varanus* blood.

A series of parallel experiments was carried out in which the flies in each of a pair of boxes, after their infecting feeds on the same animal at the same time, were nourished for the ensuing 2 or 3 weeks on *Varanus* blood and on clean monkey blood respectively. The number of infected flies in the *Varanus* series was 4 in 364 (1.09 per cent.); in the monkey series 10 in 328 (3.04 per cent.).

In another similar series of paired experiments in which the flies were nourished on *Varanus* and fowl blood respectively, the figures were: for *Varanus*

blood, 13 infected flies in 407 dissected (3·1 per cent.), and for fowl blood 24 in 404 (5·9 per cent.). The total percentage of infected flies in all those nourished on *Varanus* blood was 3·7 per cent. (19 infected in 507 dissected).

It is doubtful whether these differences are really significant, and that any inhibitory effect is exerted on the trypanosomes by *Varanus* blood.

With the baboon similar parallel experiments showed a still smaller disparity, and it is highly improbable that any inhibitive effect is exerted by baboon blood on *Trypanosoma gambiense* or *T. rhodesiense* developing in *G. palpalis*.

In man's blood in the tsetse, only the shorter form of the crystal has been observed, and as a rule only rarely, sparsely and irregularly. Occasionally, however, they are fairly numerous, although never present in anything like the quantities or the size found in baboon's blood. It would appear that the bloods of different individuals differ in the amount of crystals they yield during digestion in the fly; some produce none at all. On the other hand the irregularity may be due to failure to examine the fly at the stage of digestion most favourable for the deposition from human blood.

It is interesting to note that the crystals do not occur in the digesting blood of any of the four species of monkeys that have been tested. Of these the mangabey is relatively resistant to trypanosomes, though far less so than the baboon. Nor do they occur in tabanids or *Stomoxys* fed on ruminant blood.

As to the nature of these crystals, no attempt has yet been made to study them beyond observing that they are insoluble in ether. It is remarkable that the two animals whose blood is richest in the substance responsible for these curious deposits are to all intents and purposes completely resistant to infection with the polymorphic trypanosomes by any normal route.

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ANNOUNCEMENTS.

OFFICERS OF THE SOCIETY.

At the first meeting of the new Council, held at Manson House, on the 27th June, 1935, the following appointments were made:—

Honorary Treasurer.

Dr. OSWALD MARRIOTT was appointed Hon. Treasurer for the ensuing two years in place of Sir ARTHUR BAGSHAWE who had been elected President.

Honorary Secretaries.

Dr. C. M. WENYON and Dr. N. HAMILTON FAIRLEY were re-elected Hon. Secretaries for the ensuing two years.

Local Secretaries.

Forty-four Local Secretaries were re-elected for a further term of two years. The following new Local Secretaries were appointed, subject to their acceptance of the Council's invitation: Dr. R. BRIERCLIFFE, *Ceylon*; Dr. SEVERO MAR, *Mexico*; Dr. A. H. B. PEARCE, *Fiji*; Colonel J. A. SINTON, *India*; Mr. R. SENIOR-WHITE, *India*; Dr. O. VAN STENIS, *Venezuela*; Dr. G. FRASER, *Assam*; Dr. H. G. EARLE, *Central China*; Dr. H. D. WEATHERHEAD, *British West Indies*; Dr. L. J. WEBB, *Zanzibar*. Dr. H. C. DE SOUZA-ARAUJO was invited to represent the Society for the whole of *Brazil* instead of North Brazil only.

OPENING MEETING OF THE NEXT SESSION.

The opening meeting of the 29th Session will be held at Manson House, 26, Portland Place, London, on Thursday, 17th October, 1935, when the new President, Sir ARTHUR BAGSHAWE, *C.M.G.*, will deliver his Presidential Address.

MANSON HOUSE FUND.

Mrs. ALICE CHALMERS has given a further donation of £100 to the Manson House Fund.

TRANSACTIONS
OF THE
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AND HYGIENE.

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Proceedings of the Opening Meeting of the Session held
at Manson House, 26, Portland Place, London, W.1,
on Thursday, 17th October, 1935.

Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*,
in the Chair.

PRESIDENTIAL ADDRESS.

PROBLEMS OF HEALTH AND DISEASE OF SOME SMALL
TROPICAL ISLANDS.

BY

SIR ARTHUR G. BAGSHAWE, *C.M.G.*, M.B., D.P.H.*

I have long thought that interesting results would emerge from a study of disease in small tropical islands, especially those diseases which require for their spread an intermediate non-vertebrate host. Many such islands have, or had in the past, infrequent contact with other islands or the larger land masses and few of the inhabitants leave them in the course of their lives; there is in fact little migration to and fro, so that clinical observations can be relied upon to give a picture of the diseases or parasites peculiar to the island in question. When therefore it fell to my lot to write a Presidential Address I collected a large number of references to malaria, filariasis, schistosomiasis, etc., in such islands. I found, however, that the available information was fragmentary, in fact so incomplete that to put it on paper would make dull reading, though it would

* I wish to acknowledge the help in writing this address given me by my old colleagues at the Bureau of Hygiene and Tropical Diseases, especially Captain SHEPPARD and Miss COVENTRY.

form a useful collection of facts for a future survey. I have selected therefore for this address what seemed to me most interesting among my notes and though I cannot hope to say much that is new to you I hope to present my matter somewhat of a fresh aspect.

Malaria.

I begin with the Lesser Antilles and with a small island which is well known and not remote, Barbados, which has lately come into the limelight owing to its invasion by malaria.

Barbados (population, 167,000) is somewhat larger than the Isle of Wight and is highly cultivated; it has been a British possession since 1625. The island is of coral and water disappears rapidly over the greater part of it except in the rains which fall from July to January.

Within the memory of living man Barbados has been free from malaria but the reason was not apparent till Ross's great discovery in 1897. In 1901 one of our Past Presidents, Dr. G. CARMICHAEL LOW (1901) reported that *Anopheles* could not be found on the island, a conclusion in which the entomologist LEFROY concurred; Low noted the presence of a swamp 3 miles from Bridgetown, the capital, in which *Culex* was breeding. In a paper read at a meeting of the British Medical Association Low (1913) attributed the absence of anopheles to the island's isolation (it is 78 miles east of St. Vincent, its nearest neighbour), the distance of the above-mentioned swamp from town and harbour, and to the fact that vessels lay in an open roadstead a mile from the shore. In the same year MALCOLM WATSON (1913) fresh from his experience in Malaya, visited the island and attributed the absence of anopheles to the want of suitable breeding grounds; it has since appeared that he was not far from the truth.

In 1927 a fever became epidemic in Barbados and was soon recognized to be malaria. The first case was identified on 8th October. In November there were 250 cases under treatment and by 16th December the number rose to 853, all except three of the eleven parishes being affected. In the next year, 1928, there were 2,951 cases reported with 94 deaths; in 1929 a smaller number, 560 cases with 40 deaths (L. C. HUTSON, 1930); and after 17th October, 1929, no further cases, so that the epidemic subsided after a period of two years. Similarly the bacteriologist reported in 1929 subtertian parasites in 170 out of 885 blood examinations and in 1930 no case of parasites contracted in Barbados. In the *Annual Report of the Chief Medical Officer for 1931* it is stated that no anopheline larvae had been found since January, 1930. If the dates given are correct it would appear that malarial transmission ceased 3 months before the larval forms of anopheles disappeared.

At the outset Dr. SEAGAR (1928), Lecturer in Tropical Hygiene at the Imperial College of Agriculture, Trinidad, was called to the island and reported the presence of *Plasmodium falciparum* and over 1,000 cases of the disease. He identified *Anopheles albimanus* which he found to be breeding freely,

especially in hollows in the fields holding water, with grass growing up from the bottom; these would be transient collections. From their distribution he concluded that the anopheles had been there many months; they had probably been introduced by schooners carrying fruit, and here it may be noted that HANSCHALL (1928) on one occasion at Bridgetown found mosquitoes of undetermined genus in the forepeak of a schooner.

On the epidemic being reported in London a serious view was taken by the Colonial Advisory Medical and Sanitary Committee which pressed for the appointment of a special mission to deal with it. The House of Assembly, however, was not prepared to find the funds, and thought that local action would be sufficient. Antilarval measures were indeed undertaken but there is divided authority in Barbados, the lay leaders were sceptical of the need for vigorous action, and much less was done than the experts thought necessary. However, the disappearance of malaria was lasting. Some five years after the subsidence of the epidemic, HASLAM stated that malaria and anopheles were absent. The mosquitoes had failed to make good their footing. HASLAM (1935) writes—"Both indigenous malaria and its conveying mosquito continue to absent themselves from Barbados. The continued absence of the anopheles, like its disappearance in 1930, must be attributed, along with many other advantages of this fortunate island, to the kindness of Providence rather than to the sanitary efficiency of man. . . ."

This, however, is not the whole story. T. W. M. CAMERON (1929) has told us that there was malaria in Barbados at the end of the 18th century, and in perusing the annual medical reports for the island I find the following interesting passage from the pen of J. HUTSON (1914), Public Health Inspector:—

"It is probable that malarial fevers occurred here as late as the middle of the eighteenth century at any rate. HILLARY, a pupil of BOERHAAVE, the celebrated Dutch physician, practised in the island from 1752 to 1758, and kept a careful monthly record of meteorological conditions and the various epidemics that occurred. During these years he describes two or three outbreaks of malarial fever, and he says 'I must observe that intermitting fevers, especially quartans and tertians are very rarely or never seen in this island now, unless they are brought hither from some of the Leeward Islands, or some other places which are less cultivated, and not yet cleared of the woods; though it is said that they were more frequent here before this island was cleared of its wood and cultivated.'

"HILLARY's explanation of the gradual disappearance of malarial fever is probably correct, and a similar diminution of malaria resulting from cultivation has been seen in England and tropical countries, the haunts of the mosquito carriers of the disease being gradually abolished.

"The date of the last cases in Barbados is not known, and it is remarkable that the disease has never been re-introduced. It is interesting to observe that although the haunts of anophelines appear to have been destroyed by cultivation, there yet remain numerous collections of water, ponds, marshes, casual pools in the rainy season, which in other countries would be typical breeding places for anophelines, especially the permanent ponds more or less covered with water-lilies."

HUTSON continues:—

"Re-introduction would appear to be inevitable. For generations past numerous small craft of from 50 to 100 tons have traded between Barbados and adjacent malarious countries and colonies, going up the Orinoco to Bolivar, or lying in the rivers of British,

French, and Dutch Guiana, returning here with cargoes of firewood, charcoal, and such commodities, and discharging on the wharves of the inner harbour within the town. It is a matter of scientific interest to ascertain whether or not anophelines actually arrive in these vessels at Barbados.

"The experience at Khartoum has been that importations of anophelines by river boats and steamers, after mosquitos had been abolished in the town, was not infrequent. (BALFOUR)."

I have consulted HILLARY's book (1766) and feel that I am insufficiently familiar with the description of malaria by the old writers to be sure of the accuracy of the diagnosis ; these old authors wrote a language which it is difficult for us moderns to understand. He writes for instance :—

"The weather continuing to be wet and cool, several were seized with an irregular, ingeminated [redoubled or repeated], intermitting quotidian Fever ; which at the first generally put on the appearance of a continual remitting Fever, but in two or three Days' time usually changed to an ingeminated Quotidian, with all the symptoms of that Fever, as usual in England." After treatment which is described, "the Fever was generally carried quite off by a critical Sweat on the Seventh or Ninth Day ; but in some few it came to intermit regularly after that time and then was soon cured by the *Cortex Peruv.*"

If it be accepted that this was malaria it would appear probable that malaria is not new to Barbados, and that under exceptional conditions *A. albimanus* or other anopheline vector can temporarily establish itself, though this event is infrequent.

The subject of changes in the distribution of malaria, especially that of fresh invasions, is an interesting one and I propose to go a little further into it.

Of the West Indies HIRSCH (1883) writes :—

"Among the West Indian islands those chiefly affected by malarial sickness are Cuba, Jamaica, San Domingo, Guadeloupe, Dominica, Martinique, St. Lucia, Grenada, Tobago and Trinidad ; while others such as Antigua, St. Vincent and Barbados enjoy a relative immunity. . . . In the Bahamas malarial fever is comparatively rare ; in the Bermuda group it is almost unknown."

Instances are given of malaria in the two last groups in the sixties and seventies. CAMERON (1929) writes that malaria is now endemic in all the islands except Montserrat and St. Kitts. Of the three islands described by HIRSCH as relatively immune, in Antigua, according to McDONALD (1922) there is no question now of such an immunity. In one year he saw 672 cases and he states that it has a very serious effect on the quantity and quality of the labour supply ; the predominant form is the subtertian. Here it would appear that conditions have altered since the period antecedent to HIRSCH but whether an anopheline was introduced we have no means of knowing. In St. Vincent the 1933 report records 14 deaths from malaria, and an earlier report mentions 532 notifications, so that this island also cannot now be described as relatively immune.

HIRSCH gives an instance of the fresh appearance of malaria in the Dutch East Indies :—

"One of the islands of the Indian Archipelago, Amboina, had until the year 1835, enjoyed a remarkable immunity from malarial sickness, but in that year a severe epidemic

said in consequence of an earthquake that took place at the time, and since then has been a permanent seat of pernicious malarial fever and has consequently one of the most unhealthy places in the East Indies."

have been unable to get information of the present malarial state of Aldabra. The *Encyclopaedia Britannica* speaks of it as "comparatively

free from the introduction of malaria to Mauritius is well known. The island is believed to have been free from the disease till 1865 when malaria became epidemic and in Port Louis itself out of 80,000 persons 22,000 perished in 18 months. It is assumed that *Anopheles gambiae* was introduced about that time. In any case this mosquito was later found there and later still (1923) a malarial carrier, *A. funestus* (MACGREGOR, 1923), as well as *A. maculipalpis* a competent host. Whatever be the true history, malaria has since remained one of the chief problems of Mauritius (J. BALFOUR KIRK, 1934).

Another instance of the introduction of malaria has recently been made known by MATTHEW (1931) and HERMITTE (1931) in Aldabra, an outlier of the Seychelles in the Indian Ocean.

There are four groups of coral islets which form dependencies of Seychelles ; the group in question is 630 miles S.W. of Mahé, the largest island of the Seychelles group, all of which have had the reputation of being malaria-free.

In 1908 proved malaria occurred in Aldabra, an atoll with a long axis of 10 miles and a short axis of 5 to 10, with a land rim averaging a mile in width ;



ALDABRA ISLAND.

Produced from L. C. D. HERMITTE's paper in the *Records of the Malaria Survey of India, 1931, Vol 2.*

Permanent population appears to number 100 and the occupations are coconut growing and turtle catching. The outbreak followed the arrival of labourers from Possi Bé, Madagascar, and lasted 10 months ; 91 cases occurred in July and August. It was confined to the settlement on one of the four sections of

the atoll. Search was made for anopheles, but without success, by Dr. POWER and Mr. J. C. F. FRYER (1910), Shuttleworth Research Student of Caius College, Cambridge, and now of the Ministry of Agriculture, who was in that region studying coral formations. FRYER's search lasted from October to January and he wrote :—" I feel confident it (*Anopheles*) does not exist on Aldabra." He went so far as to suggest passive transmission by *Stegomyia* of which there was no lack.

Malaria was not heard of again in Aldabra till 1930. In July and August of that year HERMITTE in Mahé, the main island of the group, saw patients with fever from Aldabra and Assumption Islands (Assumption lies less than 50 miles away). Four of the Aldabra patients had crescents in their blood, so that the diagnosis was free from doubt. An employee was sent to collect larval and adult mosquitoes ; he despatched larvae which proved to be *A. gambiae*, and later adults were obtained. The mosquitoes were found to breed in rocky pools containing from one inch to two or more feet of rain-water ; failure to find larvae on Assumption Island was attributed to most of the pits being dry. On the second occasion then both the malarial parasite and the transmitter were demonstrated and the question arises whether anopheles were there between the epidemics, that is from 1908 to 1930, or were on each occasion introduced. Either explanation is credible. Apart from the fact that the atoll has a rich fauna of birds, large tortoises and wild goats on which the anopheles might exist, the permanent population is small and, given infrequent introduction of infected persons, anopheles might feed on the islanders for long periods without becoming infective. On the other hand both Aldabra and Assumption are served by steamers whose water arrangements are described as primitive, so that introduction of mosquitoes would not be difficult. FRYER's failure to find *A. gambiae* in the dryer part of the year finds its counterpart in many areas of tropical Africa.

HERMITTE criticizes the measures taken to keep anopheles out of Mahé. Cargo, he says, is discharged into lighters and conveyed in these to the customs shed on the pier which connects the shed with the town. *After this*, some 24 hours later, the cargo is fumigated with sulphur in a shed with open eaves. Thus mosquitoes have more than a sporting chance of getting away. Probably this procedure has now been rectified but, since unintelligent methods of this kind are still met with, it seemed to me worthy of mention.

Another instance of the recent introduction of malaria is that of Grand Comoro, a French island between the north of Madagascar and the mainland of Africa, described as a huge volcanic mass with an area of 1,200 square kilometres and a population of 75,000. There is abundant rain but the water runs through the porous soil ; rain-water therefore is retained in cisterns. Prior to 1924 all reports had said :—"No malaria owing to absence of anopheles and lack of water."—RAYNAL (1928) visited the island to report on an epidemic which had raged for two years and was then subsiding. The epidemic was described

as "grippe." The mortality was 15 to 30 per cent. RAYNAL found all three types of malaria, subtertian being most frequent. He points out that malaria was not entirely new to the island for it has long existed at certain ports in communication with other islands of the group and Madagascar.

Several causes are suggested for the epidemic, the chief being that the enhanced price obtained for vanilla had at once brought infected persons from Réunion and produced such wealth that the owners called for more and more cisterns, in which the anophles bred, for here, unfortunately, the possession of cisterns denotes a man of consequence. Another interesting suggestion is that the discontinuance of the Messageries Maritimes steamers had caused increase of the sea traffic by "boutres," or dhows as we should call them, which may have brought anopheles. At any rate malaria was unknown at the time of the French occupation (1886). The species of anopheles is not named.

Here a few words may be said about another small island in the Indian Ocean, Rodrigues, 365 miles from Mauritius. All the conditions seem suited to anopheles, streams, swamps and marshy pools and a profusion of mosquitoes but no *Anopheles*. Their introduction would seem to have been prevented by the barrier reef which compels vessels to lie a mile from the shore. MACGREGOR (1923) and BALFOUR KIRK with ANDRÉ (1933) have reported separately on this island. A proposal to build a jetty to enable vessels to discharge cargo without the intervention of lighters was rightly opposed by MACGREGOR as likely to bring to an end Rodrigues's freedom from malaria.

Two instances have been given in which *Anopheles gambiae*, that "dexterous colonizer" as Colonel ALCOCK described it, has invaded islands. It has also performed the feat of crossing the Atlantic.

In 1930 SHANNON (1930) reported the presence of this mosquito on the American continent in Natal, the nearest port in South America to the continent of Africa and lying north of Pernambuco. Later PINTO (1931) confirmed this finding and described his investigations of the epidemic which followed in Natal, which is described as unprecedentedly severe. Of 172 mosquitoes dissected 62.8 per cent. were infected and 30.2 per cent. had sporozoites in the salivary glands. The mosquitoes were found also 182 kilometres away. Here *A. gambiae* must have been introduced either in the swift packets which cross weekly from Dakar to Port Natal, requiring only 4 days for the transit, or in aircraft; the first alternative is favoured. Later SHANNON (1932) returned to the subject and gave a description of forty-two Brazilian breeding grounds. These are found to consist of small shallow collections of seepage and surface springs usually fully exposed to the sun; in ponds, streams and marsh waters anopheline larvae were found, but not *A. gambiae*. SHANNON tells us that up to 1930 [?] aircraft had made the South Atlantic crossing on eight occasions, most of them starting from Dakar or its vicinity, and landing at Port Natal. It took Bert Hinkler, in 1931, 22 hours.

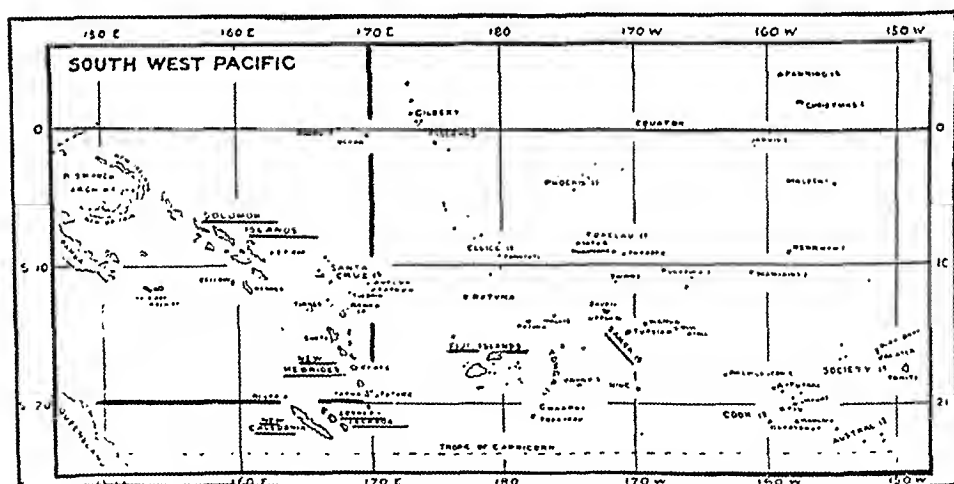
The introduction of *Anopheles gambiae* to South America is a fact of practical

as well as zoological importance; it is the first known case, SHANNON writes, of a species of anopheles pertaining to one faunal region being introduced into another. He discusses how far *A. gambiae* is likely to spread in South America and expects its distribution to be patchy. It is, however, a very efficient malarial host, and since it is not restricted in its chances of breeding as in a small island and may firmly establish itself in the moister regions, the effect might be almost as harmful as its introduction to a non-malarial country. It is clear too that if *A. gambiae* can survive 4 days in a steamer or a shorter time in aircraft, can cross a width of ocean of 2,000 miles, and establish itself on the other side it is hard to set a limit to the possible extension of this dangerous species.

One is inclined to speculate how long it will be before malaria appears in some of the South Sea Islands, now free. BUXTON (1927) has indicated the limits in Melanesia of *A. punctulatus*, the malaria carrier of the Pacific:—

"South and east of the New Hebrides," he writes, "anopheles is absent and no malaria occurs in the Loyalty Group, New Caledonia, Fiji or any part of Polynesia. One may summarize the matter by saying that *Anopheles punctulatus* and malaria occur eastward to 170° E. and southward to 20° S."

LAMBERT (1928), in discussing this subject, wonders why anophelines have not been carried on from the New Hebrides and Solomon Islands to the neighbouring islands of Fiji, New Caledonia and the Loyalties. In modern times, he says, for over one hundred years boats of all descriptions have passed freely



SOUTH WESTERN PACIFIC.

The thickened lines of Latitude S. 20 and Longitude 170 E. represent approximately the southward and eastward limits of Malaria in the South Western Pacific.

Reproduced from P. A. BUXTON's *Researches in Polynesia and Melanesia*.

between these groups and in the old days the custom was to fill fresh water barrels from streams and springs near the beach for all vessels, which would seem an almost certain way of transferring anopheline larvae, but the fact is

that *A. punctulatus* has not been so transferred, or if it has been, it has not become established.

Notwithstanding, it is necessary to keep a close watch as BUXTON emphasizes. After noting that an excellent example of a temporary breeding place for *A. punctulatus* may be found at Vila, the port in the malarial New Hebrides, he writes :—

“ It is to be remembered that not only the health of Vila is at stake. *A. punctulatus* is not a specialist in its breeding places and it would easily establish itself in Fiji or Samoa especially as there are two swamps close to the quay at Suva, Fiji, and almost in the main street of Apia and Pago Pago, Samoa.”

There are on the other hand islands where an efficient malaria transmitter is found, human carriers are usually present but no indigenous disease results. Such in the West Indies are the isles of St. Croix or Santa Cruz in the Virgin Isles, an American possession since 1917, and that of St. Barthelemy, a French island near the Virgin Isles group but administered from Guadeloupe. LOWMAN (1929) tells us that *A. albimanus* was found in St. Croix (population 11,000) in a careful survey by Lieut. HAYES, many infected Porto Ricans come in but the island is “ practically malaria-free.” St. Barthelemy, TARA (1933) states, has a population of 2,384, almost entirely white. The predominant insect is *A. albimanus*, and infected persons come in from Guadeloupe. Nevertheless, of 400 children examined in two successive years only four had splenic enlargement, and when the author is called to a case of malaria it has nearly always been contracted in Guadeloupe. It seems improbable that there is any indigenous malaria. No doubt the absence could be explained if we knew all the facts. One of the chief industries of St. Croix is stated to be cattle raising and it seems possible that the cattle provide blood for the anopheles, but *A. albimanus* is a notorious transmitter (DARLING and others), its blood-thirstiness is generally remarked and it is described in the Canal Zone as semi-domesticated, occurring everywhere about houses and villages.

Filariasis.

I started with Barbados, and will now add a few words about filariasis in that island though the facts are fairly well known.

Barbados was formerly notorious for elephantiasis, as the name “ Barbados leg ” indicates. In an examination of the night blood of 600 persons from the hospital and private sources Low (1901) found 12·6 per cent. infected with *Microfilaria bancrofti* and 4·5 per cent. had pathological changes indicative of filarial disease. Of 100 *Culex* taken in the wards and corridors of the hospital, 23 per cent. harboured filariae in various stages of development. So that at that date the percentage of filarial infection was high. HUTSON (1921) gives a table of admissions to the Barbados Hospital from 1900 to 1919-20 for elephantiasis and filariasis. For the first 9 years the average admission figure was 27·6. Mosquito regulations were then made, a sanitary inspector appointed and the average for the next 5 years was 22. The next step was supervision of the

inspectors, following which the figure over 7 years was 6.1. Whether there was any other possible explanation of the drop does not appear and here may be noted the tendency to attribute such improvement to measures of sanitation rather than to more natural if obscure causes. SILER (1915) attributed the decrease of elephantiasis to measures against *Stegomyia fasciata* initiated in 1908, the result of which again would be reduction of all domestic mosquitoes. Whatever be the reason manifestations of filariasis are now infrequent in Barbados. HASLAM (1935) writes :—" Filariasis and filarial elephantiasis figure very inconspicuously in medical experience in Barbados now-a-days."

Of another West Indian island, Grenada, LOW (1913) said that with apparently ideal conditions of climate and intermediaries filarial infection was practically non-existent. And MACDONALD (1917) stated that *Culex fatigans* was common, but filariasis "in an acute form" was unknown; no microfilariae were found in 1,000 blood smears. Later reports only confirm these observations.

Rectal Schistosomiasis.

An infection which is of particular interest in the West Indies is rectal schistosomiasis.

Thanks to Drs. CAMERON and JONES we have a fairly complete account of this in the island of St. Kitts. St. Kitts is 68 square miles in extent, was settled in 1623 and from it the rest of the Leeward Islands were colonized. CAMERON (1928, 1929) visited the island in 1928 and studied the schistosome infection reported by JONES. He found it to be restricted to the area of two permanent streams, as had been shown in 1923 by MUENCH, and made the important discovery that the infection is shared by monkeys. These creatures, *Cerco-pithecus sabaeus*, the West African green monkey, were introduced long ago as pets; and during the French wars escaped to the mountains and multiplied greatly, living in packs. Five out of seven of these monkeys were infected with *Schistosoma mansoni* and one had a severe dysentery.

It appears that the same monkey is found in Nevis, Grenada and Barbados, but fortunately *S. mansoni* is not present in these islands. One writes "fortunately" because the presence of this infection in wild animals renders it impossible, even if it were practicable, to clear the island by treatment of the human inhabitants.

Since CAMERON's article I have seen no reference to his observations and I call attention to them because they seem of considerable interest and importance.

JONES (1932) has reported one-fourth of the population of St. Kitts to be infected, but this appears to be an over estimate. He has found planorbids emitting bifid cercariae, probably *Planorbis guadeloupensis*, and these would appear to be the mollusc hosts.

Other West Indian islands in which rectal schistosomiasis is found are St. Martin, Antigua, Montserrat, Guadeloupe, Martinique and St. Lucia. MANSON's first patient (1902) is believed to have come from Antigua. The

infection seems to be quite common in the two French islands, and it is by no means always manifested by symptoms. Thus NOC (1910) in Martinique found ova of *S. mansoni* in 37 out of 225 persons and in an orphanage in 32 out of 45. Lateral-spined eggs were first noticed in natives from Guadeloupe in 1906; these were under treatment for dysentery in Martinique. CLEMENT in Guadeloupe found 20 per cent. of 1928 stools to contain the ova and MARQUE has recorded the large proportion of 150 out of 223 boys and 93 of 159 girls as harbouring them—a total of no less than 64 per cent. There is no information about the snails in these French islands.

There can be little doubt, says STIRR, that schistosomiasis was one of the diseases introduced from Africa with cargoes of slaves. Quoting BUTLER he writes:—"It is reasonable to think that during 292 years of slave trade between 1512 and 1804 every type of disease that the continent of Africa might boast of was brought to the West Indies." We must assume that the bladder infection was also introduced but died out as did sleeping sickness for want of a suitable intermediary host.

Guinea-Worm.

Another parasitic infection which came into the West Indies with the African slaves was guinea-worm. HIRSCH writes:—

"According to the unanimous opinion of the medical authorities for Guiana, Brazil and the West Indies the dracunculus was imported into these countries of the New World by negroes from the West Coast of Africa; and it has almost disappeared again from them, excepting at one or two small centres since the importation of negroes has ceased. One of these centres is the island of Curaçao . . . in which it is said there are still cases of dracontiasis occurring somewhat frequently among the native population."

The reference given is BUSK, *Med. Times*, 1846, May* and a note is added that POP, writing from Curaçao in 1859, makes no mention of the disease.

At first sight it seems remarkable that guinea-worm should have been reported in Curaçao as late as the 40's for the slave trade came officially to an end early in the 19th century. However, Sir HARRY JOHNSTON states that, between 1828 and 1878, 50,000 negroes were released from slave ships off the West Coast of Africa by the British Navy. We may assume that at least as many slave ships got away undetected, so that Africans may well have been received in the Dutch West Indies as late as 1845.

When the supply of slaves ceased guinea-worm disappeared, for its invertebrate host is absent. This is a sufficient explanation and one that should serve for the absence of guinea-worm from the Dutch East Indies also. However, it does not. BRUG tells us that infected persons from India and Arabia have been coming in for centuries and *Cyclops leuckarti* is present, but guinea-worm

*The passage in question is as follows:—

"In America the guinea-worm is unknown, except in persons who have had communication with Africa or other parts where it is indigenous. The island of Curaçao is the only locality in the New World offering an apparent exception to this fact, which it would be highly desirable to ascertain the real state of in this instance."

infestation is very rare. Four imported cases have been described and one doubtful autochthonous case. In a laboratory experiment BRUG infected cyclops readily with larvae obtained from an Arab; the infected crustaceans were fed to a gibbon which, when killed a year later, had a full-grown guinea-worm (67.5 cm.) in the left calf. There seems then to be no reason for the absence of human infestation provided that infected cyclops get into the drinking water. The freedom of the archipelago from guinea-worm is in fact attributed to the preference of the inhabitants for running water for drinking, since in water of this type the crustacean host is not found; and ROUBAUD is quoted as stating that where in French West Africa the natives drink running water guinea-worm cases are absent—a good instance of the effect of native custom in determining the presence or absence of a disease.

In a book entitled "*A treatise of the diseases most frequent in the West Indies and herein more particularly of those which occur in Barbados*," by RICHARD TOWNE and dated 1726, that is over 200 years ago, I find:—

"The countries where this Distemper is discovered, are very hot and sultry, liable to great Droughts; and the Inhabitants make use of stagnatory corrupted Water, in which it is very probable that the ova of these Animalculæ [guinea-worms] may be contained."

The Island of Saba.

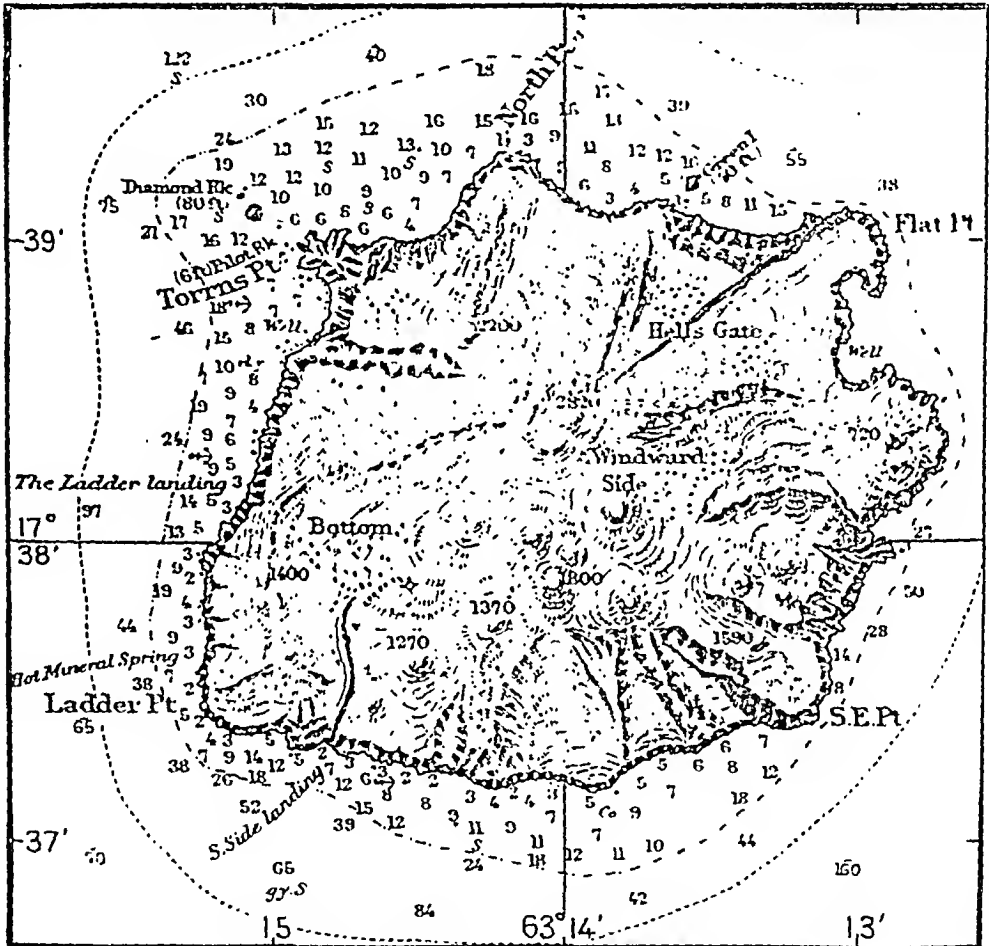
Exercising the PRESIDENT's privilege to discuss what he likes I now leave the subject of disease and turn to a very interesting example of acclimatization of the white race in the tropics. There is a small island not far from St. Kitts in which white people have maintained not only purity of race but also their physical and mental vigour for 250 years and only now by force of circumstances are being crowded out by their black neighbours. PRICE (1934) has written a valuable paper on the subject and I draw extensively from him.

The Dutch island of Saba, lying between the Virgin Isles and St. Kitts, is described by PRICE as a young volcanic island, some 5 square miles in area, fertile and verdure-clad, rising in a tangle of rugged hills to a peak of 2,887 feet.

The history of its colonization is obscure, but it appears to have been settled from the neighbouring island Statia before the middle of the 17th century by private persons under concessions from the Dutch West Indian Company. Saba is of special interest for us because the British captured it in 1665, and with one interruption held it till 1680, and it is still English-speaking. In 1665 there were 87 Hollanders, and 54 English, Scots and Irish, with 85 negroes and Indians, and the records state that the defeated Dutch settlers were sent to the island of St. Martin, so that the whites from that date hailed chiefly from the British Isles.

The island is not easy of access, there are two regular landings where loads are carried up rugged paths, one of which is called "Ladder," by men and donkeys to the lowest and principal settlement named "Bottom" at 800 feet. The donkeys are unpopular because they are the cause of unemployment. The total population is given as 1,447 persons, but according to the *Encyclopaedia*

Britannica it was, in 1911, 2,387. The people are mixed, white and black, but one cliff village, at 1,660 feet, of 231 persons is almost entirely white. Inbreeding among the whites is considerable for there is no intermarriage with the blacks. The extent of it is indicated by the record that there are now 292 Hassells, 149 Johnsons, 95 Simmonses, 58 Sagors and 52 Everyys; the name Hassell first appears in the records in 1672, that of Simmons in 1687. But inbreeding has



SABA ISLAND.

(From U.S. Hydrographic Office, Chart No. 1011, 1914), reproduced from A. GRENFELL PRICE's paper in *The Geographical Review*, 1934, Vol. 24.

not destroyed fertility, stamina or ability. Families of eight or more children were common in the 19th century. The white Sabans are agriculturalists, and sturdy workers, carrying loads up gradients which no donkey could manage. As to ability it is stated that during the Great War no less than 95 officers and one quartermaster of the United States Navy were Saban born, surely a remarkable record for such a small island. In only one isolated village of 30 to 40 persons is there evidence of degeneracy. The purity of the race is an uncontested fact.

The women are said to be the handsomest of the West Indians and to be distinguished by their slimness and fresh colour—so the *Encyclopaedia*, but PRICE tells us that the health of the women is much inferior to that of the men because from the traditions of the slave days they cannot work in the fields, nor become housekeepers, nurses or domestic servants; they spend their time indoors making Spanish lace. PRICE was favourably impressed with the white school children who were alert, well-dressed and clean. Writing of the cliff villages which are mainly white PRICE says :—

“ The cliff villages are picturesque, clean, and well kept, like most of those in the Dutch possessions. The pretty little wooden houses are for the most part painted white, with green or brown shutters and red roofs. They average four or five small rooms but often contain as many as eight or nine. The Sabans use very substantial frames of hard wood set in firm foundations. They reinforce their buildings wherever possible and guard all windows with solid wooden shutters as protection against hurricanes. Most of the houses stand in small plots, which are kept spotlessly clean and are often cemented and which, like the narrow, winding, but well kept streets, are bounded by stone walls. These last, when of cut stone or fine masonry, usually date back to the slave days. From most roofs wooden gutters carry the water to one or more cisterns, though some cisterns are supplied from the concrete yards that surround the greater number of the houses. These reservoirs are of immense value. There are more than 250 of them in Saba, and shares in them pass by will. Nearly all the houses of the whites have pit privies, which seem fairly satisfactory.”

The climate of Saba is tropical. The temperature at Bottom ranges between 71° and 80° F.; the rainfall is from 1,000 to 1,200 millimetres. There is no malaria and “ few filarial or other tropical affections ” and there is no trace of hookworm. Many white Sabans live to a great age. PRICE points to the great disproportion between the women and men—the census of 1932 showed 342 men, 655 women, 233 boys and 219 girls; that is of the adults counted 65 per cent. were female, and 34 per cent. male. But the disproportion is explained by the enterprise of the young men. They go abroad to increase the family resources and spend only brief periods in the island, which leads to what PRICE calls “ economic birth control.”

Between 1860 and 1920 two blows ruined the white settlers. The first was the emancipation of 700 Saban slaves, after which production became difficult and the price of labour rose. The second blow was the change from sails to steam. Most of the energetic young men were drawn away to New York and other centres where they secured positions as commanders and officers of steamers. Thus the island lost her slaves, schooners and vigorous young people, so that the white population to-day consists largely of aged persons. Moreover, Saba is clearly turning coloured as are almost all the islands and borderlands of the Caribbean. White settlers cannot compete with prolific negro families in which men, women and children are all workers and prepared to accept a lower standard of life than the white. Scientific medicine is hastening the process by improving the vital statistics of coloured people. At present white and coloured are in about equal numbers.

The conclusion reached by PRICE from his survey is that cold-temperate-

zone whites can retain a fair standard for generations in the trade-wind tropics if the location is free from the worst forms of tropical disease and if the economic return is adequate and the community prepared to undertake hard physical work, but eventually such a community will fall before the economic competition of a coloured people.

It seems a sad pity that such a race should disappear, or at least be scattered. The negro's "disregard of sanitation, his miserable cabins, his dirt and carelessness and his neglect of the good 'white' houses he so frequently occupies in Bottom" contrast sadly with the standards of the people he is supplanting.

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COMMUNICATIONS.

GENERAL HEALTH CONDITIONS OF CERTAIN BEDOUIN TRIBES IN TRANS-JORDAN.

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I. INTRODUCTION.

The desirability of conducting a brief investigation into the health conditions of certain of the nomadic and semi-nomadic tribes of the area lying to the East of the Jordan valley, arose from observations made by the Officer Commanding Desert Patrol and from repeated reference regarding their unsatisfactory state of health in the various annual reports of the Department of Health of the Trans-Jordan Government.

It appeared that in recent years a combination of circumstances including the prohibition of raiding, droughts, a diminished demand for camel transport, their increased vulnerability to raids from without the territory (now happily stopped), and the depressed economic situation generally has tended to lower considerably their standard of living to a borderline condition, and that in consequence certain diseases were finding suitable conditions for spread in a soil of diminished resistance.

Special reference was made to the problem of tuberculosis, a disease whose activities are strengthened by depressed economic factors and, though definite figures were not available, the opinion expressed was that this disease was assuming formidable proportions in the casualty rate.

II. METHOD AND SCOPE OF INVESTIGATION.

The restless character of these tribes, their continuous peregrinations, their scattered camps and their singular custom of establishing these camps

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in situations inaccessible to motor transport all combined to provide difficulties in establishing contact.

Medical investigations carried out under field conditions are naturally hampered and limited in scope, but these people would succeed in baffling and perplexing the most experienced field workers. After some experience it was found most convenient with each tribe to establish camp in the first instance at the largest accessible settlement: this proved frequently to accommodate the leading sheikh, whose influence could be used to assist in the investigation. From this central camp excursions could be made by lorry to other sections or smaller groups, while if a sufficiently large community was discovered, the central camp itself was moved there. In this manner the investigation was extended to include various sub-sections of the tribes which allowed for a more widespread selection of statistics.

A certain amount of propaganda work and explanation was always found necessary to commence with. These people are shy, but at the same time proud and independent, and consequently many of them were reluctant to present themselves for examination unless they had some definite complaint. The women also were particularly reserved, and infinite patience was required before they could be persuaded to agree to examination. Generally speaking, however, it was found that after a day's work confidence was restored and men came up willingly enough, but women always presented difficulty.

In all cases a fairly thorough clinical examination was made and blood examinations carried out for malaria and anaemia. In other diseases such as tuberculosis and syphilis, specimens of sputum and blood were taken for the necessary laboratory investigations. The intradermal tuberculin (Mantoux) test was also carried out on a number of children. Records were kept only in cases where a full examination was made, though numerous other cases were seen and treated. A supply of drugs and dressings was made available for those requiring treatment, and cases requiring prolonged courses of treatment were referred to the medical officer at the nearest medical centre.

Attention was also paid to habits and customs affecting health, and an effort was made to ascertain the average food consumption of individuals.

True statistics cannot be made available, as the population of the various tribes concerned is unknown. Indeed it was very difficult to obtain even approximate population figures, and various observers ventured estimates differing by many thousands.

III. TRIBES CONCERNED IN THE INVESTIGATION.

Time was limited, and the investigation was confined to examining a sample number from three large nomadic and semi-nomadic tribes—the Howaitat, Beni Sakhr and Beni Hassan. A short investigation was also made amongst a section of the Sirhaan.

The history of these tribes is vague and indefinite and relies mainly on traditional accounts.

(1) The *Howaitat* would appear to have occupied the Southern part of Trans-Jordan for several centuries and it has been conjectured that they may be descendants of the Nabataeans who held the caravan road to Yemen and had their capital at Petra ; in which case their association with the country is an ancient one. They are now a semi-nomadic tribe with a probable population of about 10,000, whose fortunes have declined in the last decade from causes already referred to early in this report.

(2) The *Beni Sakhr* probably had their origin in the Hijaz and are said to be descended from one of its most powerful tribes, Harb. They migrated north probably several hundred years ago. Their history affords every indication that they were a powerful fighting tribe. Latterly, they have taken some interest in agriculture and own lands in the Balkaa district. They number probably about 12,000.

(3) The *Beni Hassan* also originally came from Hijaz and have been in Trans-Jordan between two and three hundred years. They are now no longer nomadic and are more or less settled in the Zerka River area where they cultivate extensively. Their population which is estimated at about 14,000 is said to have greatly decreased in recent years through drought and famine.

(4) The *Sirhaan* were known as a powerful tribe of the Hauran in the latter half of the sixteenth century, but owing to internal strife leading to desertion of some of their sections, they were driven out and settled for some time in the Wadi Sirhaan area. Pressure again forced them to move and their wanderings included various parts of Trans-Jordan and Palestine for many years. The tribe was settled by the Turks some years before the War where they were given land to cultivate west of the railway between Deraa and Mafraq. In 1925 they were heavily raided and lost a great deal of their flocks and property. They are still semi-nomadic but are poor and depressed ; and they now number about 1,500.

(5) It is generally admitted that recent years have seen a decline in the prosperity of the various tribes under review, and that in some instances conditions have sunk to levels bordering on complete destitution and starvation. In the writer's brief experience among them, evidence corroborating these surmises was not far to seek, and can be backed by figures which are recorded later on in this report. That health conditions have deteriorated in consequence cannot definitely be proved by actual figures since no previous medical statistics are available, but it is indisputable that such depressed conditions lower resistance to disease generally, and to certain diseases in particular, of which mention will be made under their respective sections.

More intimate contact with civilisation is not an unmixed blessing. While certain benefits, such as education and to some extent health, are put within the reach of these people, the many evils they absorb outweigh the advantages.

Socially they cannot compete with the more sophisticated, nor would they desire to do so unless they were forced into it by circumstances. The internal combustion engine is rapidly replacing their "ships of the desert"; their traditional occupations of raiding and fighting are no more; they are more directly exposed to certain diseases notoriously associated with civilisation, such as tuberculosis and syphilis; contact with town life, politics, cinemas etc. are undermining tribal discipline, and many of the younger people will eventually become detribalised and derelict. They are being relegated to a particularly uncertain agricultural future, while they never were and probably never will be really good agriculturists, at all events not good enough to compete with their village neighbours who have been engaged in such work for generations.

The sentimental side of their life is disappearing and their proverbial hospitality and chivalry will disappear with it.

IV. HABITS, CUSTOMS AND OTHER CONDITIONS AFFECTING HEALTH.

A few brief notes regarding certain customs and habits of the tribes and other factors affecting their health conditions seem relevant to this report. It is unfortunate that it is chiefly the bad habits and customs which come under review, for they have many pleasant customs and habits worthy of note but not concerned with health.

(1) *Sanitary Conditions*.—Little or no attention is paid to sanitation and the simple principles of hygiene are not understood. The sanitary accommodation provided by the wide open spaces is, however, probably the most suitable for these people. Adults usually relieve themselves at some considerable distance from the camp, sunlight and the dry soil assisting in sterilising the dejecta. On the other hand, the young children do not respect the claims of modesty and exercise their functions at any convenient spot within the camp. Consequently considerable human contamination exists, especially if the tribe has been stationary for any length of time. Animal contamination is, of course, always present in and around the tents. The frequency of tribal movements is the saving grace and on that account gross insanitary conditions seldom develop.

The effect of these sanitary deficiencies is probably reflected to a considerable extent in the frequent outbreaks of enteritis, particularly among children, which contribute considerably to the high morbidity and mortality rates. Intestinal worms are naturally unrestricted in their activities.

(2) *Contact with Animals*.—All the tribes live in close contact with their animals. Camels, goats, sheep, horses, donkeys, dogs and fowls have the freedom of the camp and many of them share the sleeping quarters of their owners. It is indeed fortunate that these animals harbour so few diseases known to be communicable to man. *Ascaris* infection is prevalent and it yet

remains to be proved whether tuberculosis is a disease of goats and camels. Outbreaks of rabies are always a possibility. The constant inhalation and ingestion of dried mixed manure together with its effect on the eyes must, however, be somewhat debilitating.

(3) *Spitting*.—The habit of promiscuous spitting is universal and is practised by all members of the community from young children upwards. From the point of view of the individual it is probably more in the interest of his own health to deposit the purulent exudates from his respiratory passages on the ground rather than to swallow them: that others may suffer in consequence does not enter into his philosophy. There can be no doubt that various respiratory infections are spread by spitting and that the increase of tuberculosis is in great measure accelerated in this way.

(4) *Communal Feeding*.—An important factor in the spread of infectious or contagious diseases is the habit of communal feeding so prevalent among the tribes. The one large food receptacle serves successive sittings of guests and relatives, and the coffee cups and tea glasses continuously move round from mouth to mouth, not infrequently encountering a subject ejecting thousands of tubercle bacilli daily. The utensils can never be clean and the fact that the floor of the tent serves as the table, allows for gross contamination of the food from dust and manure of every description.

The conditions under which food is prepared and its mode of preparation beggar description.

(5) *Cleanliness*.—Water has been from time immemorial a precious commodity, and it might be expected that its use for the purposes of lavage is not encouraged to any extent. Bodily cleanliness is not a feature among the tribes, though they appeared cleaner than many villagers seen by the writer. The dirt of the desert is, however, probably cleaner than the dirt of towns and villages and its relationship with disease is not so close in the former instance. The majority carry lice, the vectors of typhus, and outbreaks of typhus are not infrequent among them.

(6) *Clothing*.—The clothing cannot, of course, be clean, and its quality varies with the individual and his social standing. Generally speaking it may be remarked that those who can afford it wear far too many clothes. Many on the other hand were underclad from reasons of poverty.

(7) *Kissing*.—Kissing is a common form of salutation among all ages and sexes, and coupled with this may be mentioned the unpleasant habit prevalent among mothers of removing with their clothing the nasal and ocular exudates of their children. The spread of respiratory and eye conditions is undoubtedly enhanced in this manner.

(8) *The Hair*.—The hair is worn long in both sexes and is invariably infested with lice (*Pediculus humanus capitis*). These often cause irritation of the scalp, frequently leading to gross septic infection. The posterior cervical glands are commonly enlarged from these infections. A curious custom whereby

the urine of camels was universally applied as a hair strengthener and restorer was noticed in one of the tribes examined. Its effect on the growth of the hair was not ascertained, but it seemed to inhibit to some degree louse infestation.

(9) *Housing Conditions*.—The custom of living in tents cannot be prejudicial to health during the dry season, and is infinitely preferable to living in many village houses. The requirements of ventilation and lighting are undoubtedly complied with. Unfortunately many of the poorer type tents are quite incapable of resisting the elements during the winter and the occupants must necessarily suffer from exposure. The overcrowding, animal and human, which exists in the majority of tents, especially at night, is deplorable.

(10) *Tribal Medicine*.—Little information was obtained regarding tribal medicine, but it would appear that it does not exist to any great extent and cannot compare in magnitude with that of the African witchdoctor. Certain herbs are apparently used to a limited extent, and oily substances such as olive oil and *semneh* (butter). The latter is frequently used boiling to sterilise wounds.

Cauterization is universally and extensively employed for every ache and pain experienced. It is most heroic in its mode of application, and severe burning of second and even third degree was frequently noticed. It was the exception to find an individual who did not exhibit scarring of cauterization, and in one or two instances the area of scarring appeared to exceed that of normal skin. There can be no doubt that this art is practised to excess and that frequently severe sepsis results.

The treatment of syphilis by mercury vapour has apparently been in vogue for many years. The subject is exposed on successive occasions to prolonged contact with mercury vapour in a small tent until he becomes thoroughly mercurialised. Records of complete cure are reported and the writer was able to demonstrate negative Wassermann reactions in individuals treated in this way only, who from their history and healed lesions must assuredly have had syphilis. The toxic effects of this treatment were not reported, but much can be left to the imagination.

(11) *Water Supplies*.—Except where rivers exist water supplies are limited and scanty. They vary very much according to the locality and the season of the year. Springs, wells and cisterns were the main sources seen and generally speaking the water appeared fairly satisfactory. In many instances water has to be carried long distances; and this naturally tends to restrict its use for washing purposes.

(12) *Climatic Conditions*.—With the exception of the Jordan Valley, the country enjoys a good climate. The relative humidity is low, and the extremes of temperature not unduly trying.

Certain of the nomadic tribes in their migrations escape the worst of the winter which is probably somewhat severe on tent dwellers. The poorer classes with miserable tents and limited clothing must suffer considerably from exposure during the colder months, and pneumonia is reported to be a frequent cause of

death at that time. The high day temperatures of summer are compensated for by comparatively pleasant nights. The low rainfall is the most important feature and, while it may have little direct influence on the health of the individual, it has its repercussions on the health of the community indirectly by producing poverty, distress and hunger.

It is not proposed here to give meteorological data which in any case could not be applied accurately for nomadic peoples.

V. DIET AND NUTRITION.

The food supplies of the Bedouin have no doubt been an uncertain proposition from the dawn of their history. Methods of replenishing depleted larders were formerly, however, more numerous than they are in these days of law and order, and it is unlikely that famine conditions were permitted for any length of time.

The last few years of drought have wrought havoc with their meagre agricultural efforts which even normally are barely sufficient to keep body and soul together. An enquiry into their present food supplies revealed a state of affairs pitiable in the extreme, and there can be no doubt that many from the poorer sections are on the verge of starvation. Probably numbers have already died from starvation or from conditions accelerated by it. This statement applies to all the tribes under review (probably in lesser degree to the Beni Hassan who have recently been in receipt of organised relief), but particularly to the small section of the Sirhaan.

During the course of the investigation individuals were closely questioned regarding the nature and quantity of their food supply, and from the information received a rough estimation may be made of its deficiencies. Its main constituent is *khubs* (Arabic unleavened bread), and among the poorer classes who are in the majority it would appear to be the only constituent for the greater part of the year. For those who possess goats and camels, milk products such as *semneh* and *lebban* may be available in small quantities for a short season during the year. Olives may be consumed to some extent during their season and small quantities of grapes, figs, melon, dates and tomatoes, etc., are also seasonal products for the few who can afford them. Meat is available only on special occasions or when guests are being entertained, and as the animal slaughtered has to provide for numerous successive sittings of the tribe the amount consumed is negligible. Coffee and tea are usually available, the latter having some food value from the amount of sugar added to it.

A great majority of those examined were, however, living on bread and bread alone, anything else being considered a luxury. Further, many were existing on very small quantities of bread averaging perhaps between 400 and 500 grammes daily. The food value of this commodity which has been worked

out by WILSON for the Egyptian prison authorities in drawing up a prison diet is expressed as follows:—

	Grammes
Gross protein per 100 grammes	6.7
Available protein per 100 grammes	5.0
Biological value of available protein	2.0
Available fat per 100 grammes (gross less 5 per cent.)	0.95
Available carbohydrate per 100 grammes (gross less 5 per cent.)	47.5
Gross calories per 100 grammes, 242.	
Available calories per 100 grammes, 224.	

The vitamin content is not stated, but it probably contains small amounts of B₁ and B₂.

An adult consuming between 400 and 500 grammes daily would, therefore, receive about 1,000 calories or about a third of what is considered to be the average dietetic requirements of the individual.

Further, this particular bread is neither palatable nor easily digestible, nor does it contain the main dietetic requirements, protein, fat and carbohydrate, in the proportions required for a balanced diet. The diet may be said to be grossly insufficient in quantity, to be deficient in mineral salts and vitamins, to be lacking in both energy value and biological value and to be improperly balanced. Granted that at certain seasons of the year, some fruit and milk may be available, the quantities are negligible and can have little influence on the value of the diet as a whole.

From these observations, it can be confidently assumed that the majority of these people are suffering from malnutrition, have a lowered resistance to disease generally, are the potential subjects of a group of deficiency diseases, and are incapable of sustained labour.

Much has been written in recent years about the diet of the African native and its deficiencies, and attempts have already been made by various Colonial Governments to improve it. In the opinion of the writer who has had personal experience of various East African dependencies, the diet of even the poorer African tribes is infinitely superior in quantity and quality to that of the Bedouin.

A rough classification of their nutritional condition was made during the investigation, those examined being divided into categories of good, fair and poor. It was obviously impossible to carry out height-weight ratios, and the classification did not aim at scientific accuracy, but consideration was given to development, muscle tone, etc. This was coupled with an estimation of anaemia, through haemoglobin tests carried out with a Tallqvist haemoglobin book. The results are shown in the appendices on pp. 245 to 248. It will be seen that out of 1,030 people examined only 164 or about 16 per cent. could claim to be well nourished, while 348 or about 33 per cent. were definitely poor. Many of the latter provided examples of extreme emaciation. Of the good groups

it will be noted that the majority are in the neighbourhood of the ages 20 to 40, and on enquiry it was frequently found that many of these had been away at work of various kinds and had been consequently in a position to provide themselves with more adequate rations.

VI. ANAEMIA.

The majority exhibited various degrees of anaemia and it was only in very rare instances that an individual approached the normal (100 per cent.). From a study of results shown in the appendices it will be seen that the average taken all over is between 60 and 70 per cent.

It was observed that this anaemia is not associated with any particular disease, though naturally those suffering from malaria and other blood destroying diseases must claim a share in it. It was a noteworthy feature throughout the whole community irrespective of disease. There can be no doubt but that it is due mainly to deficiency in a diet which has already been shown to be lacking in blood forming elements. The degree of anaemia is fairly constant throughout the tribes with the exception of the Sirhaan who show a lower figure, probably attributable to the high incidence of malaria among them.

VII. PRINCIPAL DISEASES.

Diagnosis in the field is handicapped by many factors, not least being the extraordinary difficulty of obtaining from the individual information regarding the history of his condition. Every query is met by vague and evasive replies, or by long-winded laborious statements usually entirely irrelevant. The same remarks apply in seeking information regarding family histories, death rates in families, etc. The medical officer is consequently left to his own resources and has to base his findings on the clinical manifestations of a single examination. As a result correct diagnosis is possible only in the very obvious conditions, which constitute but a small proportion of the actual diseases existing. In this investigation, records were made only where there could be little doubt about the diagnosis, and much that was of medical interest had to be omitted. The figures submitted therefore do not by any means represent the actual incidence of disease.

(1) *Tuberculosis*.—Diagnosis in this disease had to be made principally on history and clinical findings. Fortunately the Bedouin are very clear on the question of an important feature of this disease namely haemoptysis. They apparently realise its significance, and give decided and definite replies to requests for information on this point.

The main features of the pulmonary variety of this disease are, with certain modifications, similar to those found elsewhere, and need not be elaborated here. The figures provided show only those cases in which a combination of symptoms and physical signs left no doubt as to the diagnosis in the mind of the examiner. As a result, the figures probably represent the more advanced

cases of the disease, and many of the early or suspected cases are not included. Regarding the extra-pulmonary variety, the same remarks apply and only the obvious lesions are included.

It will be seen from the summary that the figures point to a particularly high incidence of this disease among the tribes. For example, taking the pulmonary variety: out of 356 Howeitat examined, 52 or 14.6 per cent. were affected; from 317 Beni Sakhr, 48 or 15.1 per cent.; from 273 Beni Hassan, 22 or 8 per cent. and from 84 Sirhaan, 13 or 15.4 per cent. The number of Sirhaan examined, 84, is too small to be of any statistical value, but the figures show that the incidence of the disease is probably fairly high among them. The non-pulmonary variety is distributed fairly evenly among the tribes.

Regarding the sexual distribution of pulmonary tuberculosis, out of 773 men examined 92 or 11.9 per cent. and out of 257 women 43 or 16.7 per cent. were affected, a considerable difference which, from the information obtained, may be due in some measure to the strain of successive pregnancies at an early age amongst the under-nourished.

While the well known clinical varieties of tuberculosis were recognised, it would appear that the disease is more acute and runs a more rapid course than that of Western Europe. This seems to be the opinion of the majority of medical officers in Trans-Jordan, who frequently see cases of this disease at the various medical centres in the country. They describe in many instances a rapidly progressive condition with a fatal termination, corresponding to the infantile type in Europe. It is unfortunate that there are no figures available for death rates due to this disease among the tribes, but from the evidence of this investigation and the high incidence observed, mortality rates must be alarming.

Regarding the non-pulmonary variety, Table I shows the types encountered.

TABLE I.
EXTRA-PULMONARY TUBERCULOSIS—NUMBERS OF CASES.

	Beni Hassan.		Beni Sakhr.		Howeitat.		Sirhaan.	
	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.
Glands	—	4	3	—	—	—	—	1
Spine	1	—	—	3	4	—	—	—
Joints	2	3	2	—	2	1	1	—
Bones	—	3	—	2	1	2	—	—
Abdomen	—	—	1	—	1	—	—	—
Skin (Lupus)	—	—	—	—	1	—	—	—
Totals	3	10	6	5	9	3	1	1
Grand Total	13		11		12		2	

The impression obtained was that non-pulmonary tuberculosis was also to some extent a more acute disease than in Europe. Particularly was this the case in connection with spinal caries of which several cases were seen. One particular instance which may be quoted is the case of a leading sheikh of the Howeitat tribe, who had Pott's disease of the mid-dorsal region. He gave a history of only a few months' duration, was partially paralysed when examined, and died before the writer left Trans-Jordan.

The diagnosis of extra-pulmonary tuberculosis is, as in the case of the pulmonary variety, severely handicapped through the lack of radiological assistance, and many suspected cases had to be omitted, so that here again the figures under-estimate the true position. Angular deformity of the spine, matted groups of enlarged cervical glands with old sinus formation, chronic sinuses leading to joints, such as the hip and knee, and certain miliary types were usually included as being diagnostic. Abdominal tuberculosis was suspected in many cases, particularly in children, but only two have been recorded as being definite.

The intradermal tuberculin test (Mantoux) was carried out on a number of children of ages ranging from 5 to 15 years. Unfortunately the results of this test have to be observed not less than 48 hours after inoculation, and it was found very difficult to persuade the children to return a second time. Many of those inoculated failed to put in an appearance again, and the numbers are, therefore, small. However, certain figures are available as will be seen from Table II. The test was carried out with 0.1 c.c. freshly prepared P.T. (B.W. & Co.) in a dilution of 1 in 1,000 carbolised normal saline.

TABLE II.

Age in years.	Number Examined.	Number Positive.	Age in years.	Number Examined.	Number Positive.
5	27	7	12	21	13
6	21	4	13	7	2
7	13	1	14	6	1
8	7	3	15	2	1
9	3	0	16	1	1
10	17	6			
11	12	4	Totals	137	43 = 31.3 per cent.

The results are of interest in showing that tuberculous infection is probably fairly widespread among the tribes and that a fair proportion of the younger generation are potential subjects of it. It is also interesting to note that while the percentages of positives in the Howeitat, Beni Sakhr and Sirhaan were about the same (between 35 and 40) that of the Beni Hassan was only 14 per cent. Reference to the appendix shows that the tuberculosis figure for Beni Hassan was considerably lower than the other three, the figures being Howeitat 17.9 per cent.,

Beni Sakhr 16 per cent., Sirhaan 17·8 per cent. and Beni Hassan only 12·8 per cent. It seems apparent that there must be some relationship between the two findings, which points to the fact that the Beni Hassan are less heavily infected than the other three tribes.

Whether or not the bovine bacillus is concerned to any extent remains a subject for future investigation. Cows' milk is not consumed and the little amount of goats' or camels' milk is usually boiled before use. In any case the question of tuberculosis in goats or camels might be worthy of investigation.

The general conclusions regarding tuberculosis cannot be based on comparative figures for other countries, since the results cannot be statistically accurate. It is, however, quite apparent that the tribes are heavily infected, and tuberculosis is among them a disease contributing in great measure to their morbidity and mortality rates. How long the disease has been among them it is impossible to state. From their history it appears possible that they have been in contact with the disease for generations. That contact, however, has been slight and cannot be compared with the mass contact of villagers and town dwellers. For that reason, it is highly probable that the tribes have developed little racial tolerance or resistance to the disease, a fact which would account for the acute type frequently seen in adults. It follows, therefore, that the incidence of the disease is likely to increase for a time, particularly now that they are more in contact with civilisation, and that their bodily resistance is at such a low ebb. The outlook appears grave and requires serious consideration.

(2) *Veneral Diseases*.—Syphilis did not appear to be a serious problem among them. It was confined more or less to certain families, one member of whom at some time or other had become infected and had passed it on to his wives and children. All individuals examined were questioned regarding a history of this disease, and those giving suspicious information were requested to produce their families for examination. Samples of blood for a Wassermann reaction were taken in all cases in which lesions suggestive of this disease were observed, in cases giving a possible history and in the families of the aforementioned.

Mention has already been made of the tribal treatment of this disease by mercury vapour which is undoubtedly effective in some instances.

The principal lesions met with were mucous patches in mouth and throat, iritis, papular and circinate skin syphiloids, laryngitis, various forms of osteitis associated with bone pains, perforation of the palate, various genital ulcerations and scarrings, and destruction of the nasal cartilage (most frequent type). Blood for examination was taken in 75 instances (including cases and contacts) and was found positive in 20 and doubtful or weakly positive in 7. The disease was not confined especially to any one tribe but was, as already mentioned, a disease of certain infected families, with a few exceptions among men who had probably acquired their infection extramaritally in the towns.

The Bedouin are now coming to understand the value of anti-syphilitic treatment which can be obtained at the various district health offices, and many cases investigated had already received courses of treatment. The tendency among them is to terminate their course of treatment as soon as they feel the benefit, and long before the disease is eradicated. This is the principal weakness in the prevention and control of this disease, and could be countered to some extent by propaganda methods and by mobile dispensaries.

Gonorrhœa was not observed, though in a few instances it might have been suspected from the history obtained. It probably exists to a limited extent among men who have been away at work in towns. The report of the Director of Health, Trans-Jordan, for 1933, states that only 213 cases of syphilis and 75 cases of gonorrhœa were noted during the year in the whole country. Most of these are from the towns and larger villages, so that avenues of infection are within reach.

As long as the tribal system prevails, it is unlikely that venereal diseases will increase to any great extent, and in any case they do not constitute a serious problem from a health standpoint.

(3) *Diseases of the Eye*.—The eyes were examined in all cases, and treatment and medicine dispensed for conditions requiring it. Special attention was paid to the incidence of trachoma and a summary of findings is shown in the appendices. Trachoma in its various stages is seen to be a disease of considerable endemic importance amongst the tribes, its relative frequency being greatest amongst the Beni Sakhr (173 cases in 317 examined), 54.5 per cent.; and least amongst the Beni Hassan (54 cases in 273 cases examined), 19.7 per cent.

Other eye conditions encountered were various forms of conjunctivitis and keratitis, leucoma, pterygium, cataract, corneal ulcer, trichiasis, pannus, blepharitis, etc. At the time of the investigation, conjunctivitis did not exist in epidemic form.

Blindness was comparatively infrequent, taking into consideration the high incidence of eye disease. In the 1,030 examined, complete blindness was noted in only three cases, the blindness of a single eye in six.

The incidence of eye disease would undoubtedly warrant further and more extensive measures for prevention and treatment than are available for the tribes at present.

(4) *Malaria*.—The natural tendency of the tribes in their migrations to establish their camps near water would at first sight appear to indicate that malaria would be endemic among them. This is not the case. With the exception of the Sirhaan who were in the throes of a severe epidemic, malaria was not prevalent to any extent. This is borne out by the result of blood examinations which were carried out in all cases and by the spleen rate which is shown in the appendices. Of 1,021 blood examinations only 43 were positive, 27 of these being from the Sirhaan.

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The antimalarial work of the Trans-Jordan Health Department is, despite lack of sufficient credits, most thorough and effective, and a study of the report of this department for 1933 shows how the incidence of malaria has decreased to a considerable extent in recent years. Those who had malaria among the tribes almost invariably gave a history of having resided for a period in the Ghor or Jordan Valley, where antimalarial measures to be effective would cost a fortune. It will be seen, however, that despite the antimalarial measures carried out by the Department of Health Trans-Jordan, certain groups and individuals in the various tribes by reason of their movements may from time to time be exposed to malarial infection.

It is highly desirable that those infected should receive thorough courses of treatment, not only for their own sakes, but for the sake of reducing what may be an increasing reservoir of infection. Individuals are unlikely to seek treatment themselves, or at all events sufficient treatment to eradicate infection, and for this and other reasons, some arrangement whereby facilities for organised treatment can be carried out seems essential.

(5) *Deficiency Diseases*.—It might have been anticipated from the information gathered regarding the food supplies of the Bedouin that the deficiency diseases group would occupy an important position in their morbidity table. During the investigation, a careful and comprehensive search was made to ascertain the prevalence of clinical phenomena suggestive of diseases of this group. The results are remarkable in that the numbers are unexpectedly small, and the types of diseases encountered by no means severe.

(a) *Scurvy*.—While symptoms suggestive of this disease were noted in a not inconsiderable number of instances, genuine severe cases were not observed. The symptoms noted were chiefly sponginess and swelling of the gums which appeared to bleed easily. This coupled with anaemia, weakness and dyspnoea might indicate a scorbutic condition and cases of this type have been recorded. The more advanced features generally described in scurvy, such as haemorrhages, necrotic areas in the jaw, oedema and nervous symptoms were not observed. The distribution of cases suggestive of scurvy, of which the majority were children, is as follows :—

Tribe.	Number Examined.	Cases of Scurvy.	Percentage of Cases.
Howeitāt	356	11	3.09
Beni Sakhr	317	15	4.7
Beni Hassan	273	3	1.1
Sirhaan	84	1	1.2

The source of supply of vitamin C (the factor mainly concerned in this condition) is a mystery, and the reason for the comparatively low incidence of

the disease is obscure. Possibly the various aquatic plants frequently contaminating their springs may infuse a certain degree of vitamin C into their water supplies.

(b) *Rickets*.—Contrary to expectations, the incidence of true rickets was practically negligible, though rachitic features were observed in a number of cases. The most noteworthy of these were various degrees of enlargement of the epiphyses of the wrists and ankles associated with pot-bellies. Advanced cases of this disease with its well known manifestations were only 6 in number, of whom 3 were Howeitat, 1 Beni Sakhr and 2 Beni Hassan. All occurred in children. While the anti-rachitic factor (vitamin D) would not appear to be present in their diet normally, the effect of sunlight, which in Trans-Jordan is plentiful throughout the year, is probably the determining factor in the low incidence of this disease. Breast feeding which is universal and prolonged is no doubt a further influence.

Pellagra, beri-beri, xerophthalmia and goitre were not observed.

The whole question of diet and deficiency diseases among the Bedouin is a most interesting one, and is worthy of further investigation. From what has already been remarked regarding their food supply, modern views of nutritional experts would appear to be somewhat laboured regarding essential requirements in diet. It is, however, impossible in this report to enter upon a lengthy discussion on this subject, however interesting it may be.

(6) *The Teeth*.—The incidence of dental caries and pyorrhoea is tabulated in the appendices. Perhaps the most remarkable feature of the whole investigation was the large number who presented freedom from dental caries. Thus we see that out of 356 Howeitat examined, 309 or 86.8 per cent. were free from dental caries, while for the Beni Sakhr, Beni Hassan and Sirhaan the figures were 82.3 per cent., 70.2 per cent. and 83 per cent. respectively. For children up to 15 years old the following table is also of interest :—

Tribes.	Number Examined.	Number with Dental Caries.	Total Number of Carious Teeth.
Howeitat	154	4	9
Beni Sakhr	154	19	50
Beni Hassan	115	13	45
Sirhaan	37	1	3

These figures certainly indicate that the tribes have escaped the ravages of dental disease so common in civilised communities, and that nutritional deficiencies apparently do not affect the issue to any extent. Probably the simplicity of their diet, and the lack of an infective agent may have an influence.

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Dental hygiene is certainly not practised and the high carbohydrate factor in their diet would suggest a very different state of affairs.

Reference to the table shows that pyorrhoea occurred to some extent in the more advanced age groups, and was extremely rare in children. It was not associated to any degree with dental caries, and frequently occurred in mouths containing a complete set of perfect teeth.

These observations on dental caries are in some respects analogous to those on deficiency diseases, and are of extreme interest and worthy of further investigation in the light of modern knowledge of the subject.

(7) *Diseases of the Ear, Nose and Throat.*—A record of tonsillar enlargement was made during the investigation and the results are shown in the appendices. Few instances of acute tonsillitis were seen, and the majority of tonsils enlarged presented various degrees of chronic hypertrophy. It is probable from the information obtained that epidemics of acute tonsillitis do occur at intervals.

Chronic otorrhoea resulting from a previous acute otitis was comparatively infrequent, the figures being as follows :—

Tribe.	Number Examined.	Cases of Otorrhoea.
Howeitat	356	3
Beni Sakhr	317	3
Beni Hassan	273	1
Sirhaan	84	—

A few cases of deafness were noted, but circumstances did not permit investigation of the causes of the condition.

Diseases of the nose so far as could be ascertained by a rather cursory examination were relatively infrequent. Adenoids were extremely rare. Cervical glandular enlargement was seldom observed in the anterior triangles of the neck, but was common in the posterior groups, the latter being probably associated with lice infestation and scalp irritation and infection.

(8) *Diseases associated with Animal Parasites—Helminthiasis.*—It was found impracticable to carry out stool examinations without additional staff and special arrangements. In any event it is very doubtful whether specimens could have been procured except from those persons suffering from some abdominal condition. A certain amount of information was obtained by questioning individuals who presented a syndrome suggestive of worm infestation. Generally speaking, the majority could describe with some degree of accuracy their own particular parasites, and they appeared able to differentiate *Ascaris*, *Oxyuris* and *Tæenia*.

The figures obtained in this way are as follows :—

Tribe.	Numbers Infected with		
	<i>Ascaris.</i>	<i>Oxyuris.</i>	<i>Taenia.</i>
Howeitat	13	12	2
Beni Sakhr	16	3	5
Beni Hassan	10	14	3
Sirhaan	4	2	—

It is unlikely that these figures represent anything approaching the incidence of infestation, since the habits and living conditions of the tribes are favourable to unlimited distribution. Particularly does this apply to ascaris infestation which is almost certainly universal. Among children it probably causes a considerable degree of ill-health, and many instances of heavy infestation producing severe symptoms were observed.

Hydatid infection was not observed, and generally speaking climatic and other conditions are unfavourable to the development of hookworm.

Rectal schistosomiasis apparently does not exist and in only two cases was urinary schistosomiasis discovered. Both gave a history of having lived at Tibouk which is apparently a known source of infestation for *Bilharzia* in Trans-Jordan.

The problem of worm infestation is not without significance from a health point of view. It is well known that ascaris infestation, when present to any extent, is productive of irritation, chronic ill-health and a consequent liability to other diseases, not least pneumonia. Preventive measures in this condition are valuable not only in limiting the infestation but from their educative effect in the principles of elementary hygiene. Treatment is particularly effective, and a combined treatment and preventive campaign would be well justified among the tribes.

(9) *Other Disease Groups of Less Endemic Importance.*—While the main object of the investigation was an analysis of the principal endemic diseases contributing to ill-health, records were made of all other diseases encountered and these are tabulated in the appendices to this report. It is noteworthy that malignant disease was not observed.

(10) *Mental Deficiency in Children.*—Not a single case of mental deficiency was seen. No doubt various degrees of feeble mindedness exist and there are a number of mentally retarded children, but in the experience of the writer, the children of the Bedouin are particularly bright and intelligent, and many were for their years more active mentally and more quickly receptive than the average European children of the same ages. It is a great misfortune that this

intelligence is not directed into the right channels and utilised in the service of the tribes themselves and for the ultimate benefit of the State.

VIII. SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

While the numbers examined represent only a small percentage of the population under review, they represent unselected groups from unselected sections of the various tribes, and may be said to give a picture of the general health conditions prevailing among the majority.

2. The tribes concerned in the investigation have been proved in the main to be suffering from poverty and consequent malnutrition, due to factors over which they have no control.

3. The food supplies of a large majority are insufficient in quantity and quality and in many instances inadequate to support life.

4. A considerable degree of anaemia, which is partly due to disease, but particularly to food deficiency, exists universally.

5. As a result of malnutrition, exposure and other less important factors, certain diseases of the endemic group are assuming proportions which cannot be regarded with equanimity.

6. Of these diseases tuberculosis is predominant. It is responsible for a considerable degree of ill-health, contributes in large measure to mortality rates and is in all probability increasing in its incidence.

7. The incidence of ophthalmic disease is high and requires an intensive campaign to counter it.

8. Malaria, though not widely endemic, represents a problem which requires attention, particularly by direct treatment.

9. Certain deficiency diseases should be controlled by general measures directed at improving the economic situation.

10. Epidemic diseases, though not prevalent during the period of investigation, have seasonal waves. Of these probably pneumonia and infantile diarrhoea take the heaviest toll in a depressed community.

11. The fact that the tribes do not readily seek medical attention until their diseases are in an advanced or even incurable state makes it imperative that the battle should be fought on their own grounds. In other words, that the benefits of early systematic treatment coupled with education in the elementary principles of hygiene should be brought to their very doors.

12. Schemes of relief, temporary and piecemeal in their application merely confuse the main issue which is to ensure that the tribes become self-supporting and permanently so. Without the practical application of this doctrine, medical and health measures merely prolong the agony.

13. It would appear to be the moral responsibility of Government, apart altogether from sympathetic or sentimental motives, to redeem some of the pledges made to the Bedouin not so long ago and to stabilise a future for them.

APPENDICES.

HOWEITAT TRIBE.

Age Groups.	Sex.	Examined.	Tuberculosis.		Nutrition.			Teeth.		Average Haemoglobin.	Trachoma.	Other Eye Diseases.	Tonsils.	Enlarged Spleen.	Other Conditions.	No. of Cases.
			Pulmonary.	Non-Pulmonary.	Good.	Fair.	Poor.	Dental Caries.	Pyorrhoea.							
0-5	M	32	-	-	1	6	14	-	-	55.2	19	6	8	-	Respiratory	41
	F	4	-	-	-	1	2	-	-	-	2	1	1	1	Digestive system	43
5-10	M	66	8	1	-	28	50	3	2	60.6	29	0	21	3	Circulatory system	10
	F	4	1	-	-	2	3	-	1	61.7	2	1	2	-	Diseases of skin	4
10-15	M	44	3	1	2	20	21	1	5	63.0	16	3	11	-	Nervous and sense organs	7
	F	4	1	-	1	1	2	1	1	63.7	3	-	2	-	Helminths	27
15-20	M	26	4	1	8	13	6	1	1	69.2	5	3	1	1	Genito-urinary	15
	F	5	1	-	3	2	-	-	1	58.0	2	1	-	-	Nutrition	12
20-30	M	56	6	1	19	24	12	5	8	70.9	10	5	7	5	Rheumatism and other general	4
	F	5	1	-	1	4	1	1	1	66.6	1	-	-	-	Joints and locomotion	7
30-40	M	35	8	3	11	23	4	8	11	72.6	7	6	2	-	Infectious and parasitic	2
	F	8	2	1	1	3	3	2	3	58.6	2	-	-	-	Ill-defined	19
40-50	M	25	10	-	5	15	3	4	2	69.0	2	4	1	1	Syphilis { Number suspected Number with W.R.+	7
	F	5	2	1	-	3	3	4	2	50.0	1	1	-	-		
Over 50	M	32	4	1	2	23	6	16	20	67.2	5	11	1	1		
	F	5	1	-	-	3	1	1	3	61.7	-	1	-	-		
Totals	M	316	43	9	48	152	116	39	57	65.8	85	47	52	11		
	F	40	9	3	6	19	15	8	12	60.1	13	5	4	1		
Grand Totals		356	52	12	54	171	131	47	69	65.3	98	52	56	12		

DENI SAKHR TRIBE.

Age Groups.	Sex.	Examined.	Tuberculosis.			Nutrition.			Teeth.		Average Haemoglobin.	Trachoma.	Other Eye Conditions.	Tonsils.	Enlarged Spleen.	Other Conditions.	No. of Cases.
			Pulmonary.	Non-Pulmonary.		Good.	Fair.	Poor.	Dental Caries.	Pyorrhoea.							
0-5	M	28	4	1	2	11	15		-	-	53.2	13	2	6	1	Respiratory diseases	45
	F	13	2	-	-	11	2		-	-	58.1	7	2	1	1	Digestive system	8
5-10	M	40	4	-	-	12	28		7	2	66.5	27	4	8	1	Circulatory system	4
	F	13	1	-	-	10	3		-	-	64.6	8	2	1	1	Diseases of skin	19
10-15	M	45	3	2	8	24	13		11	4	74.1	35	4	14	1	Nervous and sense organs	4
	F	15	1	1	-	7	8		1	-	68.1	11	3	-	2	Genito-urinary	1
15-20	M	15	2	-	4	6	5		-	3	63.9	6	3	3	-	Diseases of nutrition	16
	F	9	-	-	3	6	-		-	1	62.7	7	3	-	-	Rheumatism and other general	13
20-30	M	31	1	3	16	15			3	6	74.0	17	2	2	4	Joints and locomotion	1
	F	19	5	1	4	10	5		2	3	66.1	5	1	-	2	Infectious and parasitic	10
30-40	M	17	4	-	3	8	6		2	4	68.3	4	2	-	1	Ill-defined	4
	F	15	6	1	-	10	5		2	4	69.6	9	1	-	-	Helminths	24
40-50	M	24	6	-	6	15	3		10	16	69.0	10	3	2	-	Syphilis {	28
	F	9	3	2	1	4	4		5	8	66.4	3	2	-	-	Number suspected	
Over 50	M	14	4	-	1	10	3		11	13	71.0	6	7	-	1	Number with W.R.+	13
	F	10	2	-	1	4	5		4	7	65.5	5	5	-	-		
Totals	M	214	28	6	40	101	73		42	48	68.8	118	27	35	8		
	F	103	20	5	9	62	32		14	23	65.3	55	19	2	7		
Grand totals		317	48	11	49	163	105		56	71	67.6	173	46	37	15		

BENI HASSAN TRIBE.

Age Groups.	Sex.	Examined.	Tuberculosis.		Nutrition.			Teeth.		Average Haemoglobin.	Trachoma.	Other Eye Conditions.	Tonsils.	Enlarged Spleen.	Other Conditions.	No. of Cases.
			Pulmonary.	Non-Pulmonary.	Good.	Fair.	Poor.	Dental Caries.	Pyorrhoea.							
0-5	M	29	-	-	1	18	10	1	-	57.7	4	14	3	1	Respiratory diseases	34
5-10	F	6	-	-	1	2	3	1	-	51.4	1	4	1	-	Digestive system	14
	M	31	-	-	1	15	16	5	-	66.3	11	1	10	-	Circulatory system	16
10-15	F	5	-	1	-	2	3	-	-	85.0	-	-	1	-	Nervous and sense organs.	8
	M	37	4	-	3	23	11	6	1	68.8	7	2	6	-	Diseases of nutrition	5
15-20	F	7	2	2	2	1	4	1	-	59.3	2	2	-	1	Rheumatism and other general	11
	M	10	1	-	1	2	7	-	1	65.5	3	1	1	4	Joints and locomotion	2
20-30	F	2	1	1	-	2	-	-	-	70.0	2	1	-	-	Infectious and parasitic	16
	M	27	2	2	8	13	6	1	7	71.7	3	3	1	8	Ill-defined	2
30-40	F	14	1	1	4	5	5	1	3	65.3	-	1	2	1	Helminths	27
	M	19	3	-	11	8	-	3	8	73.6	3	1	-	1	Syphilis { Number suspected	12
40-50	F	21	3	4	2	15	4	4	7	63.8	4	4	-	3	Number with W.R. +	5
	M	15	2	-	6	8	1	9	13	68.6	4	4	1	2		
Over 50	F	7	1	-	-	4	3	2	5	66.6	1	-	-	-		
	M	27	2	1	5	20	2	19	19	70.0	5	7	-	-		
	F	16	-	1	2	9	5	12	14	67.5	4	8	-	-		
Totals	M	195	14	3	35	107	53	44	49	67.7	40	33	22	16		
	F	78	8	10	11	40	27	20	29	64.3	14	20	4	5		
Grand totals		273	22	13	46	147	80	64	78	66.7	54	53	26	21		

SIRHAAN TRIBE.

Age Groups.	Sex.	Examined.	Tuberculosis.		Nutrition.			Teeth.		Average Haemoglobin.	Trachoma.	Other Eye Diseases.	Tonsils.	Enlarged Spleen	Other Conditions.	No. of Cases.
			Pulmonary.	Non-Pulmonary.	Good.	Fair.	Poor.	Dental Caries.	Pyorrhoea.							
0-5	M	7	-	-	1	1	5	-	1	45.7	-	3	-	5	Respiratory diseases	8
	F	4	-	-	2	-	2	-	-	53.7	-	3	1	1	Digestive system	1
5-10	M	3	-	-	-	-	3	1	-	57.5	1	-	1	2	Diseases of skin	1
	F	9	1	-	1	6	2	-	-	58.8	3	2	5	6	Diseases of nutrition	1
10-15	M	9	1	-	-	3	6	-	-	61.1	3	1	3	6	Joints and locomotion	1
	F	5	1	1	-	5	-	-	-	69.0	2	-	4	2	Infectious and parasitic	36
15-20	M	3	1	-	-	3	-	-	-	68.3	-	-	-	-	Helminths	6
	F	2	-	-	-	1	1	-	-	55.0	2	2	-	2	Syphilis { Number suspected	1
20-30	M	8	2	-	4	4	-	-	-	63.7	1	1	-	2	Syphilis { Number with W.R.+	1
	F	6	1	-	2	2	2	2	2	59.1	2	1	-	4		
30-40	M	7	-	1	2	1	4	1	4	63.5	-	-	-	3		
	F	5	2	-	-	3	2	1	3	53.0	1	-	1	3		
40-50	M	4	-	-	3	1	1	1	3	70.0	3	-	-	-		
	F	3	-	-	-	2	1	2	1	53.3	2	1	-	1		
Over 50	M	7	3	-	-	5	2	5	5	67.5	4	3	-	2		
	F	2	1	-	-	-	2	1	2	60.0	1	-	-	1		
Totals	M	48	7	1	10	18	20	8	12	61.8	12	8	4	20		
	F	36	6	1	5	19	12	6	8	58.3	13	9	11	20		
Grand totals		84	13	2	15	37	32	14	20	60.3	25	17	15	40		

THE ADVANTAGES OF ATEBRIN IN THE TREATMENT OF MALARIA AMONGST CONTROLLED LABOUR FORCES IN MALAYA.*

BY

A. L. HOOPS, C.B.E., M.D., D.P.H.†

The new synthetic drugs plasmoquine and atebtrin have simplified the treatment and greatly reduced the relapse rate in malaria.

Plasmoquine, discovered in Bayer's Laboratories at Elberfeld in 1924 by W. SCHULEMANN and his associates, has been found lethal to the gametocytes of *Plasmodium vivax*, but its action on the asexual cycle of the malarial parasites is slight, and on sporozoites nil.

The evolution of atebtrin, an acridine dye, by MIETZSCH, MANSS and KIKUTH, tested in the field by PETER, MASINCEZCU, and MÜHLENS in 1930 and 1931, has, in conjunction with plasmoquine, lowered the malarial relapse rate to some 10 per cent., the cure being effected within a few days; unlike quinine or plasmoquine, which are excreted from the system within 36 hours, atebtrin can still be demonstrated in the blood and urine 2, and sometimes 3, weeks after ingestion.

It is taken up from the stomach and small intestine by the liver whence it is again excreted into the small intestine in the bile; part then leaves the system in the urine and faeces while the remainder is again absorbed by the liver: this cycle is repeated until excretion is complete.

In the blood, atebtrin is found both in the serum and in the red blood corpuscles to which it is strongly attracted, and even more so to the malarial parasites, for, when extracted from the corpuscles with alcohol, it still clings to the parasites.

Like quinine, atebtrin acts on the asexual cycle of the malarial parasite but is devoid of any direct effect on the sporozoites and gametocytes.

Testing Atebtrin on Animals and on Man.—KIKUTH and PAUL RUSSELL with bird malaria, and CHOPRA and DAS GUPTA with monkey malaria, have shown its intensity of action, while S. P. JAMES in his malarial research station at Horton has made valuable experiments with it on general paralytics.

It must be remembered, however, that birds and monkeys are not men, and that general paralytics are already suffering from a severe disease of the

* This paper was read before the Edinburgh Branch on 26th June, 1935.

† I am much indebted to Professor Dr. W. SCHULEMANN, Dr. F. M. PETER and Dr. W. KIKUTH for valuable information and demonstrations during my visit to their laboratories in June, 1935.

nervous system; also in experimental infections the conditions found in nature cannot be exactly reproduced. For this reason the practical results obtained by myself and others in Malaya in the treatment of malaria with atebtrin amongst controlled labour forces of otherwise healthy persons, are worthy of record.

Owing to the superior results obtained amongst labour forces on the rubber estates of the Malacca Agricultural Medical Board, numbering some 20,000 labourers and dependents, atebtrin has, in my practice, replaced quinine since the middle of 1932.

Some of those who continue to use quinine admit that with atebtrin there is a lower relapse rate, but they express their preference for moderate doses of quinine given for several periods of a few days each—their main object being not to cure the malarial infection at once, but to produce an increased resistance to the disease by allowing the sufferer to pass through a few mild attacks.

Incidentally, this must produce a number of reservoirs for the spread of further malarial infection.

In Malaya prevention of malaria by antimalarial measures such as regular antimalarial surveys, the upkeep of existing drainage, the draining of new seepages and breeding grounds of malaria-carrying anophelines, and when necessary periodic oiling of danger spots, is practised on many estates. By this method we aim at preventing the reinfection of the cured sufferer from malaria and we therefore do not take the risk to himself and others of trying to produce a possible immunity.

MALARIA RATES ON THE ESTATES SERVED BY THE MALACCA AGRICULTURAL MEDICAL BOARD STAFF DURING THE LAST TEN YEARS.

The Table illustrates the fall in sickness and deaths attributed to malaria since atebtrin has been used on our estates.

TABLE I.

MALARIA.

Year.	In-patients.	Out-patients.	Deaths.	Average Estate Population.
1925	2,031	—	54	18,721
1926	3,473	—	107	22,801
1927	3,352	—	108	24,081
1928	2,951	—	57	22,039
1929	1,762	—	58	25,515
1930	1,241	2,246	60	22,820
1931	934	1,877	35	21,553
1932	545	1,364	21	19,511
1933	462	542	15	19,954
1934	534	333	13	23,007

A comparison of the number of cases of malaria treated and of the malarial deaths in the past 10 years is interesting.

Until 1930 the records do not show the number treated as out-patients, but it may be assumed that they were nearly double the in-patient figure. During the first 7 years, 1925-1931, malarial deaths averaged sixty-eight a year, and cases were numerous, though falling in 1930 and 1931. In the last 3 years, 1932-1934, admissions have dropped greatly and so have out-patients, and deaths average only just over sixteen a year. The fall in 1934 coincided with a big increase in susceptible labour population.

Another good feature is that the proportion of in-patients to out-patients has increased steadily, until in 1934 on European-owned estates, out of a total of 497 cases of malaria, 483 were treated in hospital while, though the Asiatic estates have no hospitals, 51 out of their 317 cases were sent to Government hospitals.

The recent decrease in malaria is due to several causes.

(a) The decrease of labour forces in 1931 and 1932 owing to the slump : naturally the weaker members who were most liable to sickness were weeded out. This cause ceased to operate by mid-1933 : there have been few repatriations since that date. On the other hand, there has been a steady flow of immigration from India which has been intensified in the latter half of 1934.

(b) The continuance of anti-malarial measures, and especially of oiling and surface drainage which are necessary to control our chief carrier on Malacca estates, *Anopheles maculatus*.

(c) The treatment in hospital of an increased proportion of malaria cases which tends to lessen the spread of infection in the lines.

(d) The use of atebtrin for the treatment of malaria reinforced by plasmoquine, in subtertian cases, during the last 3 years—1932 to 1934.

(e) The examination of all new labour, and the twice-yearly routine examination of all labour forces, followed by prophylactic treatment with atebtrin of all who may be harbouring malaria parasites.

Treatment of Malaria on Estates Served by the Board.

Until June, 1932, the drug used both for the attempted prophylaxis and cure of malaria was quinine, usually 10 grains of the sulphate or bihydrochloride thrice daily for a week to 10 days if in hospital, or until temperature dropped if in the lines, followed by smaller doses of quinine—10 grains daily for 3 weeks after return to work, and also after the half-yearly examination if the spleen was found to be enlarged. Even under such courses of quinine a relapse rate of 50 per cent. was common, while if quinine was given only until the fever subsided the relapse rate was much higher.

In 1931, 934 cases of malaria were treated in hospitals, all with quinine ; deaths amongst these numbered 31—death rate 3·31 per cent. : in 1932, when the use of atebtrin in some cases was begun, quinine was used in an almost equal number of hospital cases : 228 malaria patients were treated with quinine, of whom 14 died (death rate 4·85 per cent.) ; while 257 were treated with atebtrin, of whom 4 died (death rate 1·55 per cent.).

In 1933, when atebtrin was in general use, of 462 persons treated in hospitals only 30 received quinine treatment, with 1 death from subtertian malaria : the remaining 432 were treated with atebtrin and of these 9 died (death rate 2·08 per cent.), 8 of them had subtertian malaria and 4 were comatose on admission.

In 1934, 534 persons were treated for malaria in hospitals : of these 33 received quinine with 1 death from subtertian malaria ; 501 received atebtrin treatment with 9 deaths (death rate 1·8 per cent.) : all these 9 had subtertian infections : 4 children died comatose in 2 hours, 4 hours, 8 hours and 40 hours respectively ; and 3 adults, also comatose, died in from 4 to 24 hours after admission. The eighth, a woman, had a subtertian malarial attack 16 days after a septic confinement and died in 48 hours ; the ninth was a cachectic case.

Routine of Atebtrin Treatment.

The method followed by me in using atebtrin is :—As soon as the patient enters hospital he is given a purgative—usually for an adult calomel, grains 3—followed by mist. alba,

2 or 3 ounces after a few hours. His blood is taken and the type of parasite determined. A dose of stock diaphoretic mixture three times a day until fever subsides helps to promote sweating and lessen distress. After the blood has been examined and a purgative and diaphoretic mixture given, the administration of atebtrin is commenced.

In proportion to age, children require bigger doses than adults. Each tablet of atebtrin contains 0.1 gramme ($1\frac{1}{2}$ grains); the dosage followed in ordinary cases is:—

Infants, up to half a tablet daily.

Children of 1 to 3 years, from half to one tablet daily.

Children of 3 to 5 years, up to $1\frac{1}{2}$ tablets daily.

Children of 6 to 10 years, $1\frac{1}{2}$ to $2\frac{1}{2}$ tablets daily.

Children of over 10 years, and adults, 3 tablets daily.

The temperature will usually fall to normal within 2 or 3 days. If the temperature does not rise again above 99° F. after 48 hours, the atebtrin treatment is continued for 5 days only. If the temperature stands above 99° F. at any time during the third or fourth day atebtrin is continued for 6 or for 7 days. Atebrin is not given for longer than 7 days in one course.

When the malaria is subtertian in type a course of plasmoquine is administered after the atebtrin treatment is completed: plasmoquine has also been given in relapsing cases of benign tertian, but not in primary cases.

The dosage of plasmoquine used for an average adult is 0.01 gramme tablet three times a day for 5 days—for a child of 10 half of a 0.01 gramme tablet three times a day for 5 days, and for a child of 6 one-third of a 0.01 gramme tablet three times a day for 5 days. Children under 6 years are not given plasmoquine without special orders.

As regards atebtrin for injection purposes, the earlier preparations were too insoluble. This difficulty has now been overcome by the production of a very soluble salt, known as atebtrin musonate. It is a dimethanesulphonate of atebtrin supplied as a yellow powder in sealed ampoules, containing 0.125 grammes each, corresponding to the single dose of 0.1 gramme of the atebtrin bihydrochloride tablet for oral use.

Each powder is dissolved in exactly 3 c.cm. of water before injection. The contents of one ampoule is the maximum single dose that should be given intravenously, but three times this amount, comprising one day's treatment, may be given intramuscularly at one time. The indications for the injection of atebtrin musonate are the presence of high or persistent fever, severe vomiting, the advent of cerebral symptoms. In addition, it may be valuable for the rapid treatment of supervised cases in the course of a great epidemic. In Ceylon it has recently been tried in this way, 0.375 grammes (*i.e.* the contents of three ampoules) being dissolved in 9 c.cm. of water and injected into the buttock daily in one dose on two successive days only. BLAZE and SIMEON claim that as a rule the fever and parasites disappear entirely after these two injections. The relapse rate, after such a short course, will probably be high.

Quinine has not been used by me in conjunction with atebtrin except in the few cases where it has been given as an injection.

Atebrin is usually well tolerated by young and old, by infants, pregnant women, nursing mothers and persons in whom other diseases are complicated by malaria. Vomiting is rarer than with quinine; most patients prefer atebtrin and many experience a sense of well-being and increased appetite during treatment.

RESULTS.

Under the treatment outlined the temperature is usually normal on the second or third day and seldom rises again above 99° F. In a proportion of cases the temperature is higher, and the parasites are more numerous on the second day than on the first day, owing to the provocative effect of atebirin on the parasites. All parasites usually disappear by the third day; and, except for crescents, none are found in any patient on discharge from hospital.

As an unusual exception, the temperature of one man with a subtertian infection remained above 99° F. for 216 hours though four injections of quinine bihydrochloride grains 10, and four injections of atebirin grains 1½ were given intramuscularly, and atebirin and quinine were used consecutively in treatment—each for 5 days.

Time Spent in Hospital.—The average time spent in hospital when plasmoquine was not administered after atebirin was 7½ days; many patients were discharged in 5 days.

When plasmoquine was given separately after atebirin the patient was sometimes in hospital for 10 or 11 days, though part of this after-treatment was occasionally given in the lincs.

Toxicity of Atebrin.

The toxicity of atebirin by itself is low as noted by the Malaria Commission of the League of Nations, and by recent writers in the *Indian Medical Gazette*.

Some 1,900 cases of malaria have been treated with atebirin on Malacca estates during the past 3 years, of which 1,207 were under my care. Amongst these latter I have observed the following:—

(a) *Severe colic.*—Nine cases of severe intermittent colic, occurred high up in the abdomen. The colic began as a rule about the end of, or within, 4 days after the termination of the course, and lasted from 2 to 4 days. Seven of the sufferers had received plasmoquine as well as atebirin. Alkalies and opiates relieved the colic which gradually disappeared.

Amongst estate staff, one manager suffering from malaria, without seeking medical advice, took double doses (six tablets) of atebirin daily for 6 days, after which he suffered from moderate colic and turned yellow. Another with subtertian malaria got severe colic after taking the ordinary dosage of atebirin combined with double dosage of plasmoquine for 4 days.

I have not included mild cases of abdominal pain such as are noted occasionally on the resumption of full diet, whether atebirin or quinine has been employed; such pain is relieved by a purgative.

(b) *Violent vomiting* is rarer with atebirin than with quinine; a case has been reported in which it occurred each time one tablet was administered, probably due to intolerance of the drug.

(c) *Severe headache*, usually frontal, is found occasionally.

(d) *Yellow staining* of the skin and conjunctivae is seen in a small proportion

of patients ; it is temporary and due to the fact that atebtrin is a dye, so that this is not really a toxic symptom.

(e) *Cerebral excitement*.—In one male Tamil with benign tertian a condition of cerebral excitement and intoxication developed on the 4th day of treatment. For the next 3 days, during which he was kept locked up, he thought his kangany was coming to beat him and he had other delusions ; he then became normal again.

(f) *Various other symptoms* have been attributed to atebtrin, such as " fits " in a patient who was afterwards found to be an epileptic ; pains in the muscles of the neck.

R. GREEN ascribes such toxic symptoms as do occur to the fact that atebtrin is eliminated slowly from the body, and so has a cumulative effect : marked toxic effects occur mostly in patients to whom plasmoquine had been given at the same time as atebtrin.

It is important that the doses of atebtrin mentioned should not be exceeded, and that the drug should not be given for more than 1 week at a time.

When plasmoquine is added to the treatment it also should not be given in excess of the doses indicated, and not for more than 1 week at a time : if possible it should not be administered until the atebtrin course is finished.

RELAPSE RATES OF CASES TREATED WITH ATEBRIN BY THE AUTHOR AND KEPT UNDER OBSERVATION FOR AT LEAST 8 MONTHS, DURING YEAR 1933 AND FIRST HALF OF 1934.

In order to arrive at a fairly correct relapse rate no case is included in Table II that has not been observed for 8 months ; many have been observed for a full 12 months.

It is to be borne in mind that some of the further attacks of malaria must have been new infections though all are counted as relapses.

Quartan malaria is not included in the table as only 17 cases have been treated with 2 known relapses.

TABLE II.
TYPE OF MALARIA.

Year.		Benign Tertian.	Sub-tertian.	Unclassified.
1933	Total observed	408	162	160
	Relapses	47	25	12
	Percentage	11.5	15.4	7.5
1934 (first half)	Total observed	219	125	85
	Relapses	24	18	5
	Percentage	10.9	14.4	5.9
				9 1 11

Period of Relapse.—In 1933, I classified separately the relapses of children and adults, and the results are given in Tables III and IV.

TABLE III.
RELAPSES AMONGST CHILDREN TREATED IN 1933.

		Benign Tertian.	Sub-tertian.	Unclassified.
Total observed	79	49	21	9
Relapses	12	9	1	2
Percentage	15.1	18.3	4.7	22.2

Amongst these children the average duration before relapse in benign tertian cases was 5½ weeks; half the relapsing cases had received smaller doses of atabrin than recommended by the makers; one of them relapsed twice—in the 6th and in the 12th week. The one case of sub-tertian child relapse occurred in the 15th week.

TABLE IV.
RELAPSES AMONGST ADULTS TREATED IN 1933.

		Benign Tertian.	Sub-tertian.	Unclassified.
Total observed	329	113	139	77
Relapses	35	16	11	8
Percentage	10.6	14.1	7.9	10.3

The first average benign tertian relapse in adults was in the 15th week; the earliest was in the 5th week and the latest in the 31st week; two cases relapsed twice, one in the 7th and 15th weeks, and one in the 28th and 35th weeks after infection; the latter showed sub-tertian parasites on the second occasion.

One pregnant woman relapsed three times in the 7th, 15th and 23rd weeks after primary infection.

Amongst eleven sub-tertian relapses in adults the average was in the 9th week, the earliest was in the 2nd week and the latest in the 19th week. Four included under the head sub-tertian were mixed sub-tertian and benign tertian infections. Two of these, both relapsing in the 8th week, showed only benign tertian parasites on relapse. Two sub-tertian cases relapsed twice, one in the 3rd and 19th week, and the other in the 5th and 19th week.

My experience in 1934 was similar. The average relapse rate of children

with benign tertian parasites was in the middle of the 5th week after infection, while with adults it was in the 17th week.

USE OF ATEBRIN ON ASIATIC-OWNED ESTATES.

Quinine is still used on a number of the smaller Asiatic-owned estates where supervision is almost impossible as there are no hospitals, and drugs are administered by a headman.

Out of 429 persons treated for malaria from Asiatic-owned estates in 1933, 210 (3 deaths) received quinine and 219 (1 death) atebrin; 1 died untreated. Only 23 were sent to Government hospitals.

Out of 317 persons treated for malaria from these estates in 1934, 141 (1 death) received quinine and 176 (2 deaths) atebrin. One woman died without treatment; 51 were sent to Government hospitals.

With atebrin the relapse rate in Asiatic estates, amongst observed cases including reinfections, was 17.3 per cent. Amongst 51 persons who were observed for 6 months, after a week's treatment with quinine, the relapse rate, including reinfections, was 77 per cent.

Blackwater Fever.—Before atebrin was in use for the treatment of malaria there were 6 cases and 4 deaths from blackwater fever on Malacca estates in 1931 and 2 deaths during the first half of 1932. In the latter half of 1932 atebrin was administered to 2 cases of blackwater fever occurring in old sub-tertian infections—one, a man, the other, a girl aged 7: both recovered. No cases of blackwater fever have been noted in 1933 or in 1934.

The treatment of malaria with atebrin is undoubtedly deterrent to blackwater fever.

BARROWMAN, working on rubber estates in the Klang district of Selangor, recently reported 90 per cent. of relapses amongst 412 cases of malaria treated with 30 grains a day of quinine for 1 week only, the rates being almost equal for benign tertian and sub-tertian infections.

Against this he reported 8.4 per cent. of relapses among 369 cases treated with atebrin only, the figures being—benign tertian 175 cases, 21 relapses, relapse rate 12 per cent.—sub-tertian 194 cases, 10 relapses, relapse rate 5 per cent.

Using atebrin and plasmoquine he found only 6 relapses in 192 cases of benign tertian and 3 relapses amongst 63 cases of sub-tertian—relapse rates 3 per cent. and 5 per cent. All his cases were observed for 6 months.

PLASMOQUINE AS AN ADJUNCT TO ATEBRIN TREATMENT.

Atebrin treatment by the eradication of schizonts prevents the formation of later generations of gametocytes. The earlier broods of gametocytes will die out in a few weeks.

Whether plasmoquine should be given to kill the latter, depends therefore on the risk of infection being conveyed to others in any particular estate. In this several factors are involved—the number of persons with gametocytes in their blood; the number of malaria-carrying anophelines present (most important), the number of bites received from infected mosquitoes; the intensity of the infection conveyed; the effectiveness of the antimalarial work. A change in the level of the surface water, an influx of unhealthy new labourers or the felling of a new area may necessitate the adoption of measures not previously needed.

The League of Nations Malaria Commission's Report (pp. 236-237) suggests that the percentage of malarial cases containing gametocytes in their blood may at times be as low as 1 per cent. though in children it may be 17 per cent. or more. But GREEN, in a series of 1,000 sub-tertian cases found that over half carried crescents in the proportion of 1 or more per 200 leucocytes and were therefore potentially infective to *Anopheles maculatus* (*Bulletin Inst. Med. Res.* No. 5, 1929). If these high rates are common in Malaya the argument for giving plasmoquine is strengthened.

GREEN found also that gametocytes seldom appeared before the 6th day in new infections and took on an average nearly 8 days to disappear under treatment with plasmoquine

compound—the limits being 4 to 13 days (*Bulletin Inst. Med. Res.* No. 3, 1929). It may be assumed therefore that the treatment with plasmoquine simplex should not be started before the 6th day in primary attacks, and that with an adult a dosage of from 0·02 gramme to 0·03 gramme daily should be continued for from 5 to 7 days.

My practice has been to give plasmoquine in all sub-tertian cases owing to its effect on the gametocytes, and in benign tertian relapse cases. There is no doubt however that plasmoquine will further lower the relapse rates if given in primary benign tertian attacks as well.

THE USE OF ATEBRIN FOR PROPHYLAXIS.

It used to be customary on most European-owned estates to give a course of quinine to all coolies and dependents found with enlarged spleens at periodic examinations of estate labour forces; most of this quinine was undoubtedly wasted; the recent *Report of the League of Nations Malaria Commission* stresses the fact that quinine, even when given in the fever-free and parasite-free period just before a relapse, exhibits a lack of therapeutic action. Also, a considerable proportion of persons with enlarged spleens, chiefly of the fibrotic type, harbour no parasites at all, and some of them have acquired an immunity.

The routine use of quinine for enlarged spleens has now been abandoned by me; atebtrin treatment has been substituted for selected cases only—especially for new locally engaged labourers who have either a recent history of malaria, parasites in their blood or a temperature. No treatment is given to those with fibrotic spleens or to any who have already received a course of atebtrin for malaria, and who show no relapse symptoms on examination.

Acting on these lines I gave a prophylactic course of atebtrin, to about half of 952 persons found with enlarged spleens at special examinations on my estates in 1933, and to a similar proportion in 1934. Those who appeared ill were put in hospital, but the majority remained at work and received a 5 days' course of three tablets daily given in one dose after they had come in from the field; no ill effects were observed.

Amongst one batch of 28 newly engaged coolies, 17 had enlarged spleens and a recent history of malaria: 7 of these, and 1 man who had not an enlarged spleen, but had parasites in the blood, and 3 others with enlarged spleen but no parasites, had a temperature. As a precaution the whole of this gang received atebtrin treatment. One Asiatic estate manager developed sub-tertian malaria after taking one tablet of atebtrin daily for 3 weeks.

On none of the Malacca estates is malaria so severe as to render mass prophylactic treatment advisable.

WALLACE, working in Kedah, finds atebtrin is the most effective drug for mass treatment at the beginning of the malarious season. In 1933, on one of his estates where malaria was seriously affecting the labour, no cases occurred within a month after a 5 days' mass course of atebtrin and the malaria rate remained low for the 2 succeeding months. On another malarious division where the parasite rate was 18 per cent. amongst adults and 23 per cent. amongst children, a 5 days' course of atebtrin and plasmoquine reduced the parasite rate to nil. After this 0·02 gramme plasmoquine was given three times weekly for 3 months, at the end of which the parasite rate was: adults 3·5 per cent., children 5 per cent. These doses of plasmoquine will inhibit the sexual forms and thus prevent infection of others.

Though no antilarval measures were taken and the malaria season was at its height, only one case of malaria occurred.

IS ATEBRIN A SUBSTITUTE FOR ANTIMALARIAL MEASURES SUCH AS DRAINAGE AND OILING?

In my opinion, "No," but it is a great adjunct to these measures. Its use is effecting a sterilisation of previous carriers of malarial infection on many

Malacca estates. A saving of oil is therefore more possible than formerly in certain areas during the less malarious months of the year.

COST OF ATEBRIN TREATMENT.

GREEN states that atebtrin is the cheapest drug for treating malaria if labourers are not subject to frequent reinfection, because it is superior to quinine both for destroying benign tertian and quartan gametocytes and for preventing relapses. It has no effect on sub-tertian gametocytes, for the destruction of which plasmoquine must be given.

In two large comparative series of cases from the same estate, GREEN found that the percentage of days spent under treatment per unit of population, including a preliminary mass treatment, was atebtrin 7 per cent., quinine 27 per cent.

With atebtrin the relapse rate was much lower, the working efficiency was maintained at a high level, and far fewer working days were lost.

During the past 3 years in Malacca, the cost of a 5 days' course (15 tablets) has varied between 72 and 89 Straits cents (1s. 8d. to 2s. 1d.). Allowing a 15 per cent. relapse rate for which 12 cents may be added, the maximum cost per head is just over \$1 Straits (2s. 4d.).

During the same period the price of quinine sulphate has varied from \$14.50 to \$17 Straits, and of quinine bihydrochloride from \$19 to \$24 Straits per pound. Taking into account the much higher relapse rates with quinine, not less than 1 ounce is needed for an average complete treatment on estates.

The additional cost of 0.15 gramme of plasmoquine given in a 5 days' course is 43 Straits cents (1s.) ; which course is advisable at any rate in sub-tertian infections whether atebtrin or quinine has been used.

While the total drug treatment with atebtrin actually costs less than with quinine in properly supervised cases, there is a still greater saving effected by the low relapse rate, lessened absence from work, and the greater efficiency following atebtrin ; the shortness and simplicity of administration is a further point in its favour.

Atebtrin is the best drug available for the controlled treatment of all types of malaria in Malaya, where effective oral administration is preferable to injection.

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A CASE OF SIMULTANEOUS INFECTION WITH YAWS AND PRIMARY SYPHILIS.

BY

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Though many cases are on record of yaws patients becoming infected with syphilis, the following case is of interest since there still persists in some quarters the belief that the two diseases are identical.

The patient was from a tribe amongst whom both yaws and syphilis are common. The two diseases have different native names; and while it is recognized that to become infected with syphilis is shameful, no such stigma attaches to yaws. Furthermore, the natives assert that in years gone by, before the Europeans came, there was no syphilis among them, but only yaws, the subjects of which disease were made to live alone in separate huts like lepers. That syphilis was brought by Europeans is probably untrue, since Arab slave-traders had established communication with Uganda many years before the advent of the white man; but these few points serve to emphasize the fact that to the native who has known all about yaws for many generations, syphilis is a new disease of recent introduction.

The history of the case is as follows. In April, 1934, there was a small yaws sore on the right shoulder for which he was treated by a native medicine man and "cured." In October, 1934, a more generalized eruption appeared on the face and on the back but this again cleared up except for two lesions

* My thanks are due to the Honourable Director of Medical Services, Kenya Colony, for permission to publish this paper, to Dr. W. WILKINSON for allowing me to report the case and to Dr. J. H. SEQUEIRA for permission to use his name.

on the back. He left his home in October and came to Nairobi where, since his wife did not accompany him, he admits having intercourse with prostitutes in native brothels.

During November his yaws rash underwent a second exacerbation and again appeared on his face. He remembered having intercourse with a prostitute at the beginning of December, and on the 24th of that month he first noticed a sore on the prepuce.

It is significant that whereas he had paid no heed to his yaws, he at once sought advice for his syphilis and was admitted to the Infectious Diseases Hospital, Nairobi. Dr. W. WILKINSON, the Medical Officer in charge of the hospital, very kindly brought the case to the writer's notice, and he was shown at the Annual Meeting of the Kenya Branch of the British Medical Association on 4th January, 1935.

There was a well-marked framboesiform yaws eruption over the head and neck which was in every way typical of the so-called secondary stage of yaws.

On the preputial margin was a small ulcer with slight puckering of its edges. The base of the ulcer was indurated and there was a small amount of clear serous discharge from its surface. A scraping from this ulcer was found on examination by dark ground illumination to be teeming with spirochaetes indistinguishable from *Treponema pallidum*. The Kahn test gave a four plus result, there was generalized enlargement of the lymphatic glands and on treatment both conditions cleared up. The primary syphilitic sore healed more rapidly than did the yaws lesions.

By a fortunate chance a small boy who was admitted to hospital at the same time, showed a yaws sore on his prepuce. The primary lesion was still present on his thigh and there was a third lesion on the shaft of the penis: and it was apparent that the two sores on the penis could have arisen from direct contact with the one on the thigh as they almost certainly did, though the child was too unintelligent to give a clear account of his illness.

It was noted that while the preputial sore in the man was an ulcer in the sense that there had been an erosion of tissue, the boy's sore was more granulomatous in character involving a heaping up of tissue, a state of affairs which is seen in all the so-called framboesiform lesions of yaws.

DISCUSSION.

Dr. J. H. SEQUEIRA, who was present at the meeting referred to above, affirmed that there could be no doubt whatever as to the nature of the sore on the penis of the man; it was undoubtedly a primary syphilitic chancre. He also stated that the lesions on the face resembled no syphilitic condition which he had met during his long experience at the dermatological department of the London Hospital.

Dr. SEQUEIRA went on to say that in 1925, when he first visited Kenya, he saw a large number of cases of yaws and he had seen many since, but he had never in his experience seen any syphilitic lesion in England or on the Continent which he might have confused with typical yaws.

It has been said that the "rupia" of syphilis resemble yaws sores, but a little care in examination will show at once that any such resemblance is exceedingly superficial. In the first place rupia are excessively rare, they are a tertiary manifestation and most intractable to treat. The "limpet-like" appearance is due to a heaping-up of dried discharge and tissue debris, and if this is removed the condition is seen to be an ulcer whose base lies below the level of the surrounding skin. There is no essential difference between the so-called primary and secondary eruptions of yaws. Histologically the two lesions are identical. They are notoriously common and by no means hard to cure. If the overlying crusts are removed the sore is still raised above the skin surface and the skin is therefore not so much ulcerated as hypertrophied.

Microscopically yaws is seen to be primarily an epidermal disturbance. The interpapillary pegs are elongated and thickened, dipping down deeply into the corium where there is a well-marked round-celled infiltration and some oedema, but without any tendency towards the characteristic endothelial proliferation so constantly seen in syphilitic lesions. The spirochaetes are found in large numbers in the deeper epidermal layers and in the superficial layers of the corium. In syphilis, of course, the organisms are found deep in the corium and the epithelium shows little change.

It would be tedious to refer seriatim to the various lesions of syphilis which are brought forward as being identical with yaws; the real answer lies in the fundamental difference in the pathology of the two diseases, a difference which is epitomised in the patient's skin by the scarring which follows syphilis and not yaws and by the occurrence of such cases as the one here described.

The carefully controlled and extensive work of SCHÖBL on Philippine monkeys has cast so much light on the problem that few can remain unconvinced of the separate identity of the two diseases.

SUMMARY.

1. A case is described in which framboesiform yaws preceded and outlived a typical primary syphilitic chancre.
2. The fundamental nature of a syphilitic lesion as ulcerative and a yaws lesion as a hypertrophy is emphasized.
3. The differences between the histology of yaws and syphilis of the skin are briefly indicated.
4. The work of SCHÖBL taken in support of such cases as that described is considered to be conclusive proof that yaws and syphilis are two separate diseases.

THE NATURAL INFECTION OF *HIPPELATES PALLIPES* LOEW WITH THE SPIROCHAETES OF YAWS.*

BY

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The possibility that yaws (*framboesia tropica*) might be transmitted by insects has been suggested from time to time, and in the literature there are several reports of the demonstration of the presence of *Treponema pertenue* in various species of flies.

CASTELLANI (1907) found *T. pertenue* in *Musca domestica* which had fed on scrapings from yaws lesions, but this method of infecting flies did not really simulate what would occur in nature. THOMSON and LAMBORN (1934) fed *Musca spectanda* on sores which had been cleaned up thoroughly to rid them of superficial pus. But ROBERTSON (1908), working at the Tarawa Hospital of the Gilbert Islands Protectorate in Polynesia, apparently made a study of *Musca domestica* infected in a natural manner. He arranged to have about 200 house-flies caught in sterilized glass jars by patients with yaws lesions when these insects came to feed on the lesions. The jars containing the flies were filled with sterile water and shaken; later the water was centrifuged. Smear preparations were made on slides from the centrifuged precipitate and stained. ROBERTSON reported that in only four of twelve slides examined did he find well-formed examples of what he called the "*Spirochaeta pertenuis* of CASTELLANI."

In the island of Jamaica, the commonest fly to be found feeding on yaws lesions and ulcers of all kinds is *Hippelates pallipes* Loew. The feeding habits of this fly and particularly the mechanism of regurgitation of a "vomit drop" has led us to suspect that this insect might be of considerable importance as a natural vector of yaws. For it is relatively easy to demonstrate motile *T. pertenue* in the "vomit drops" of these "eye gnats" after they have been fed on infectious framboesiomata.

In investigating the potentialities of *H. pallipes* as a vector of yaws, one of the first things about which we felt the need of more accurate information was the length of time that *T. pertenue* remains alive in the flies after the infecting feed. KUMM, TURNER, and PEAT (1935) found that the majority of the ingested treponemes remained motile for about 7 hours in the oesophageal diverticula of the insects, whereas the spirochaetes that entered the midgut or the hindgut lost their motility very quickly. No motile *T. pertenue* could be demonstrated at intervals of 18 or 24 hours after the infecting feed.

Other questions which followed in more or less logical sequence were: What happened eventually to the ingested spirochaetes? For how many days

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation.

after the initial meal could non-motile *T. pertenue* be demonstrated anywhere in the alimentary tract of *H. pallipes*? Was there any cyclical development of the spirochaetes in the fly, such as is known to occur with relapsing fever spirochaetes in ticks? Could any evidence be found of invasion of the salivary glands of the insects with yaws spirochaetes? Or did the spirochaetes migrate to the proboscis or anywhere else within the body of the fly? To obtain definite answers to these questions 525 flies were dissected, at least 25 specimens of *H. pallipes* were examined every day for the first 14 days following an infecting feed and the same number after 21 and after 28 day intervals.

By putting flies which had fed on infectious yaws lesions into petri dishes with a little dry granulated sugar, we have been able to keep sufficient numbers of insects alive for the periods of time required by this study. As a rule the petri dishes containing infected flies were stacked inside a wooden box for safe keeping. We have no evidence that storing the dishes in the dark inside this box had any effect other than keeping them from being bumped about on the laboratory desk.

It would obviously have been preferable in making this study to have used clean laboratory bred flies. But up to the present we have not succeeded in breeding *H. pallipes* in the laboratory in sufficient numbers for this purpose. It was therefore necessary to use adult flies caught in nature. We assumed that such flies were not naturally infected with spirochaetes resembling *T. pertenue* or with that organism itself, because of the previously reported negative results of dissections of 200 flies caught in an area where there were no infectious cases of yaws.

NUMBER OF DAYS FOLLOWING AN INFECTING FEED THAT *T. pertenue* CAN BE DEMONSTRATED IN *H. pallipes*.

In order to obtain accurate data on the eventual fate of yaws treponemes ingested by *H. pallipes*, it was deemed advisable to make all dissections by a standard technique. Thus the results obtained on successive days would be comparable.

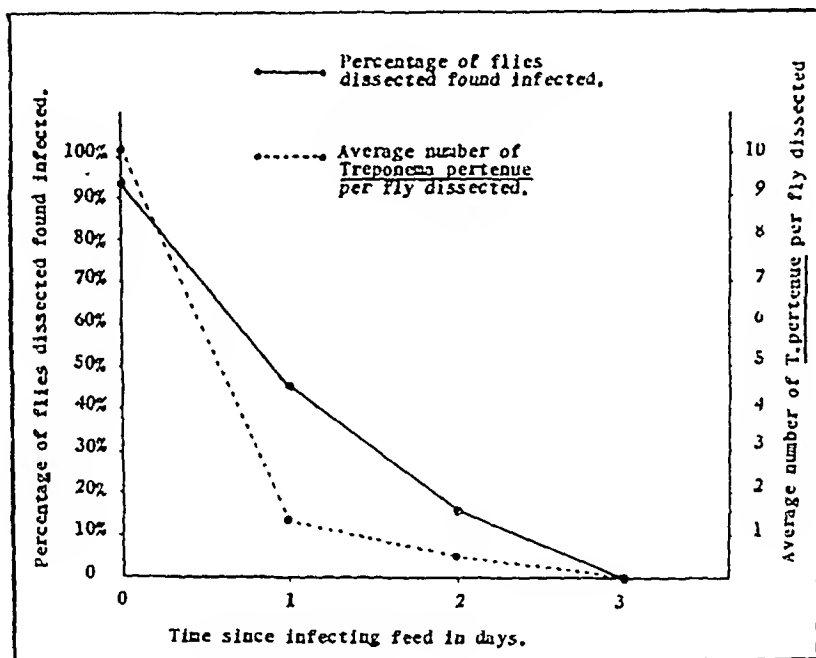
A single preparation was made of the organs of each fly, and this preparation was examined in the darkfield microscope for 5 minutes by the clock.

The alimentary tract of the fly was removed by the method previously described, except that we did not make separate preparations of the oesophageal diverticulum and of the stomach, but examined the entire digestive system in a single preparation. It was not feasible in every fly to remove the salivary glands together with the diverticulum of the oesophagus, because occasionally the lobes of the salivary glands broke loose from the crop. But it can be stated that in the large majority of the flies examined in this study, the salivary glands were included with the rest of the alimentary tract. Thus the preparation examined under the darkfield microscope consisted of the two salivary glands, oesophageal diverticulum, proventriculus, midgut, hindgut, rectum, and Malpighian tubes, together with the contents of all of these organs, mashed out under a thin coverslip. The anatomical location and appearance of these organs in *H. pallipes* has been described by KUMM (1935).

In addition to examining all of the organs connected with the digestive tract of the flies, the possibility that the treponemes might have migrated elsewhere outside the alimentary system was considered. With this in mind the probosces of the flies dissected after

21 and 28 day intervals were removed separately, and after being teased apart were mashed out and examined. Occasionally some of the thoracic muscle fibres were deliberately included in the proboscis preparation. Furthermore, any loose connective tissue from the abdominal cavity together with a few eggs and part of the oviducts and spermatheca were usually included with the preparation of the alimentary organs. Before dissecting out the digestive tract the head of each fly was cut off with a needle with a lancet point, in order to sever the oesophagus and the common salivary duct, and hence facilitate the removal of all the digestive organs in one mass.

Although it was feasible to establish a standard technique for examining all of the 525 insects used in this study, it was not possible to infect them uniformly in the first place. The reason for this lay in the fact that various batches of flies were fed on different patients and on different lesions, and even if successive lots of flies were fed on the same lesion on successive days or during successive hours on a single day, they did not all become infected to the same



degree. By pure chance alone, some flies would select highly infectious places in a lesion, where they would ingest large numbers of spirochaetes. Other flies, feeding on the same lesion but in another area, might take in serum containing no *T. pertenuis* at all or but a small number of these organisms. Therefore in each lot of flies five or ten insects, constituting a control group, were dissected and examined within a few hours of their infecting feed, to get an idea of the infectivity rate of the whole lot, that is the approximate number of spirochaetes per fly taken up in the first place.

In all, fourteen experiments were run to obtain the required number of dissections at the various intervals of time following the infecting feeds. The number of *T. pertenuis* found per fly on the day of the initial meal varied from zero to sixty-one in individual insects. And in these fourteen experiments the

average figure for the control groups of five or ten flies, dissected to obtain the infectivity rate of the lots, varied from 1.6 to 19.1 spirochaetes per fly. In all, 115 *H. pallipes* were dissected and examined on the day of their infecting feed to obtain these infectivity rates, and 1,116 *T. pertenue* were found in these flies. This gave an average figure for all of 10.1 spirochaetes per fly, but one should not assume that that figure represents the total number of *T. pertenue* taken up

TABLE I.

RESULTS OF DARKFIELD EXAMINATION OF THE DIGESTIVE TRACT OF *H. pallipes*, INCLUDING THE OESOPHAGEAL DIVERTICULUM AND SALIVARY GLANDS AT VARIOUS TIME INTERVALS FOLLOWING AN INFECTING FEED.

Interval of Time since Infecting Feed.	Number of <i>H. pallipes</i> Dissected and Examined.	Number of Flies found Infected with <i>T. pertenue</i> .	Percentage of Flies Dissected Found Infected.	Total Number of <i>T. pertenue</i> Found in these Flies.	Average Number of <i>T. pertenue</i> Found per Fly Dissected.
Same day as the infecting feed	115	108	93.9	1,116	10.14
1 day	35	16	54.7	47	1.34
2 days	25	4	16.0	13	0.52
3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28 days	25 each day	0	—	0	—

by an average fly feeding on an average infectious yaws lesion. Such an assumption would be incorrect. The figure of 10.1 spirochaetes per fly is based on darkfield examinations lasting 5 minutes by the clock and no more. It is certainly true that in the case of heavily infected "eye gnats" an examination lasting only 5 minutes does not reveal all the *T. pertenue* that have been ingested by a fly.

The graph (p. 267) and Table I show the results obtained from this study, and may be briefly summarized as follows. The number of *T. pertenue*, which could be found in *H. pallipes* following an infecting feed decreased rapidly after the day on which the flies had their initial meal. On the day following the infecting feed, less than half the flies contained yaws spirochaetes, and no organisms at all were demonstrated later than the second day after the meal on the patient. At no time could any *T. pertenue* be found in the salivary glands. Nor were spirochaetes seen in the proboscis or thoracic musculature of the flies after intervals of 21 or 28 days. And as no treponemes of any kind were seen after an interval of more than 2 days, it seemed definitely established that there was no extrinsic cyclical development of *T. pertenue* in *H. pallipes*. Presumably then, the spirochaetes were digested in the midgut and hindgut of the flies very soon after they passed over from the diverticulum of the oesophagus.

THE NATURAL INFECTION OF *H. pallipes* WITH *T. pertenue*.

With the knowledge that *H. pallipes* does not normally contain *T. pertenue* or any other spirochaetes resembling that organism, and with a fairly clear idea of what eventually happens to yaws spirochaetes ingested by *Hippelates* flies, it became possible to study the natural infection of these flies and to find out how frequently such a condition occurred.

To illustrate the manner in which *H. pallipes* fed naturally on the lesions of framboesia tropica, a photograph was taken of these flies endeavouring to obtain a meal on some yaws lesions in the popliteal space of the left leg of a small girl. Plate I is magnified about three times. Only three flies were attempting to feed on the right lower lesion in Plate I, because the scab was practically intact except for one small hole into which one of the flies had succeeded in inserting its head. A larger number of the insects were feeding in the cracks of the left lower lesion. But the upper of the three lesions showed the greatest number of all, because the scab had been broken off by the child and serum was exuding freely. The characteristic glistening black mesonota and the position of the wings of the flies while feeding is evident.*

It is quite obvious that the method used to infect flies, in which the scab is removed, superficial pus wiped off with a piece of sterile gauze wet with normal saline, and fresh serum full of spirochaetes squeezed out from the lesion beneath, while good enough in itself, involved manoeuvres that would never occur in nature. If *H. pallipes* were to be of any real epidemiological importance as a natural vector of yaws, it would of necessity have to pick up *T. pertenue* readily under perfectly natural conditions, and not only from yaws lesions carefully cleaned up by artificial means. It was therefore decided to make dissections of flies fed on yaws lesions which had not been touched or altered in the slightest manner from their original condition, to see if such flies became infected with treponemes or not.

H. pallipes flies were caught in large bore glass tubes after they had fed to repletion on various types of yaws lesions in a perfectly natural state, and they were transported in these tubes to Kingston for subsequent dissection. When the lesion on the patient as he or she came into the treatment clinic had deep cracks, or part of the scab was broken off, the flies which fed in the cracks or on the area devoid of scab were captured in preference to insects which tried to feed on the dry surface of intact scabs. After the number of flies needed for dissecting purposes had been caught, the yaws lesion was carefully cleaned up by removing the scab, wiping off the purulent secretion with a sterile piece of gauze, and squeezing out fresh serum from beneath. This serum was examined at once by the darkfield technique to find out whether the lesion studied contained many *T. pertenue* in the first place. If it did not, all flies fed on that lesion were discarded. But if the expressed serum did contain a reasonable number of actively motile *T. pertenue*, that group of flies was dissected and the percentage of them infected was recorded. Of course no flies were ever

* For Plate I the writer is indebted to the skill of Mr. DENIS M. GICK, of Kingston.

caught for dissecting purposes after a lesion had been cleaned up. Only those were used which had fed naturally before the darkfield examination was made.

In recording the results no attempt was made to determine how many *T. pertenue* were ingested by each fly. A note was simply made as to whether

TABLE II.

RESULTS OF DISSECTIONS MADE TO DETERMINE THE RELATIVE INFECTIOUSNESS FOR FLIES OF VARIOUS TYPES OF YAWS LESIONS UNDER NATURAL CONDITIONS.

Number of Lesions Examined.	Type of Lesion Studied.	Surface of Lesion : Dry or moist with Exuding Serum.	Result of subsequent Darkfield Examination of Lesion.	Number of Flies Dissected and Examined.	Number Infected with Motile <i>T. pertenue</i> .	Total Number Infected with <i>T. pertenue</i> .	Percentage Infected.
4	Primary	Moist	Many <i>T. pertenue</i>	59	25	51	86.4
1	Crab yaws	Moist	Very many <i>T. pertenue</i>	48	19	45	93.8
2	Crab yaws	Moist	Few <i>T. pertenue</i>	26	4	18	69.2
4	Crab yaws	Dry	Fair numbers of <i>T. pertenue</i>	35	3	16	44.4
10	Framboesiform on head and upper extremities	Moist	Many <i>T. pertenue</i>	112	37	86	76.8
7	Framboesiform on body and lower extremities	Moist	Many <i>T. pertenue</i>	109	32	101	92.7
3	Framboesiform	Moist	Few <i>T. pertenue</i>	28	7	17	60.7
9	Framboesiform	Dry	Fair number of <i>T. pertenue</i>	82	8	21	25.6
40			TOTALS	500	135	355	71.0



FIG. I.



FIG. II.



FIG. III.

each individual fly was infected or not, and then the percentage of the group infected was calculated. However, it may be mentioned that some flies which fed naturally took in great numbers of treponemes and others only one or two. The examination was not made for a standard interval of time. In the contents of the digestive tracts of some flies spirochaetes were found at once and in others only after a lengthy examination lasting 10 minutes or more. A single examination was made of the combined contents of the stomach and oesophageal diverticulum of each fly dissected. The presence of *Spirochaeta refringens* was recorded as well as that of *T. pertenue*, though the former observation was purely incidental to a search for the latter organism.

The results of these dissections of 500 flies which had fed on forty different lesions are summarised in Table II. Although the patients were carefully classified into groups with regard to the location of their lesions on the body, the factor of the location of the lesion appeared to be of very minor importance. In a few instances two different lesions were studied on the same patient. This was done simply for convenience and probably influenced the final result but little.

From the foregoing table it is evident that if a lesion was moist, or even if fresh serum was exuding through cracks in a broken scab, provided that this serum contained a reasonable number of *T. pertenue* in the first place, the flies would become infected. If, however, the lesion contained very few spirochaetes, or if the surface in the bottom of the cracks or where the scab was pulled up was dry, *H. pallipes* obtained few if any organisms. For example, two lesions situated on different sides of the abdomen of the same patient were studied. The first lesion was wet; so flies fed well, and 91.7 per cent. became infected. The second was dry; and of the few flies that were able to ingest a certain amount of the half dried serum, only 50 per cent. obtained *T. pertenue*.

Lesions on the perineum were usually damp, and their scabs rubbed off by friction of the opposing surfaces. Such lesions proved highly infectious for flies. What is more, the flies came to feed in great numbers on perineal lesions, when that part was exposed. Certain framboesiomata on the head and around the mouth also infected a high percentage of the insects which could be induced to feed on them. But in working on the upper parts of the body, particularly the head and arms, much time was wasted because only an occasional *H. pallipes* would come to feed naturally.

Only 27 per cent. of the flies examined contained motile treponemes. This percentage would have been higher if a long interval of time had not elapsed between infection and dissection in certain cases; but a considerable proportion of the insects were dissected many hours after their infecting feed.

Photographs were taken of two of the crab yaw lesions. Plate II shows a wet lesion which infected 93.8 per cent. of the flies which fed naturally, while only 20 per cent. of *H. pallipes* obtained spirochaetes from the lesion shown in Plate III, although large numbers of these insects can be seen crowding round the dry scabs on the boy's feet.

With regard to certain flies which were found to be infected with *S. refringens*, when they were supposed to have fed on lesions infected with *T. pertenue* only, it is likely that these flies fed twice. They probably fed first on some lesion infected with *S. refringens*, and then came to feed on the patient on whom we were working. As most of the patients were studied in conjunction with the yaws treatment clinics, such an occurrence would not be unlikely. It is but another indication that *H. pallipes* feeds intermittently on various lesions and persons. Because most of the natural infection rate dissections were made on flies caught on patients at the yaws treatment clinics, it cannot be asserted that the treponemes found in these flies always came from the identical lesion on the patient which we were studying. But as the major portion of the material ingested by each fly came from the lesion under investigation, it is likely that most of the spirochaetes originated in the same place.

Primary lesions and crab yaw lesions are particularly dangerous as a source of natural infection of flies, because they rarely have intact scabs and are usually exuding fresh infected serum in large amounts. The *T. pertenue* rate was as high as it was because, in almost all cases, we deliberately dissected only flies which had fed naturally on lesions containing at least a fair number, and preferably a large number, of yaws spirochaetes, as shown by subsequent examination.

SUMMARY.

Treponema pertenue has not been demonstrated in *Hippelates pallipes* later than 48 hours after the infecting feed, nor has any evidence been found of invasion of the salivary glands or proboscis, or of cyclical development of the spirochaetes in the fly, up to 28 days after the initial meal on a yaws lesion.

H. pallipes readily becomes infected with *T. pertenue* under natural conditions from lesions on any part of the body, though the fly feeds by preference on the perineum or on the lower extremities. Of 500 flies dissected in this portion of the study, 71 per cent. were found naturally infected.

Three important factors appear to be necessary to the natural infection of flies seeking food at a yaws lesion :

- (a) The scab should not be intact.
- (b) The surface of the lesion should be moist, or exuding serum, but not pus, freely through cracks in the scab.
- (c) *T. pertenue* must be present in abundance in the lesion itself.

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A STUDY OF THE REACTION TO PHLEBOTOMUS BITES WITH SOME REMARKS ON "HARARA."

BY

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The reaction to phlebotomus (sandfly) bites has received considerable attention because of their particularly unpleasant consequences. In Palestine the reaction to sandfly bites has often not been connected with these insects by the inhabitants, and it was considered as a skin disease, known locally as "harara" until DOSTROWSKY (1925), on the basis of clinical observation, showed that "it was nothing else than the reaction to sandfly bites" in newcomers to a sandfly country.

There are a number of clinical observations on this reaction but scarcely any experimental work has been carried out on this subject until BOYCOTT (1928) studied it with material sent to London from Jerusalem. BOYCOTT's work will be discussed below in connection with the work described in this paper.

I recently spent some months in London, studying the physiology of *Phlebotomus papatasi* in the Department of Entomology, London School of Hygiene and Tropical Medicine. This visit gave me an excellent opportunity of studying the reaction to sandfly bites of a population in which previous contact with phlebotomus could be definitely excluded.

TECHNIQUE.

Female *Phlebotomus papatasi* one or more days old were put in test tubes and placed on the skin on the inner side of the forearm. The distance from a transverse fold of the wrist was recorded and an interval of 3 to 4 cm. was left between successive experimental bites. As the reaction persisted for some weeks, there was no difficulty in recognising individual bites. Altogether about 30 persons volunteered for the biting experiments, of whom the majority had never left England. In a number of volunteers regular experiments were carried out every 3 to 8 days, for 2 to 3 months. In several cases, only one experiment was carried out in order to establish the kind of first reaction they gave. On

* I have to thank Dr. KATZENELLENBOGEN of Jerusalem for putting his dermatological material and experience at my disposal and to Professor KLOPSTOCK, Tel Aviv, for his advice in the serological questions involved.

I have also to thank all those who kindly volunteered for the rather unpleasant biting experiments.

each occasion one to twenty sandflies were allowed to bite on an area covered by a test-tube. The number of bites obtained was usually two or three times the number of sandflies in the tube.

It is necessary to define some terms used in this paper for the reactions described: the statements of former authors have often been ambiguous because they have not used clear morphological definitions for the lesions observed.

The lesion referred to as a *papule* in this paper is from 1 to 3 mm. in diameter as a rule, hard, raised and reddish; and it persists for weeks. A *wheel* is a raised, whitish, soft lesion which appears within a few minutes after the bite and disappears within one hour. The interval between bite and appearance of the lesion is termed *incubation period*.

DESCRIPTION OF THE REACTION.

All authors call attention to the needle-like pain of the bite, which is later followed by itching. But not all sandfly bites are painful, and it is often impossible to say whether a sandfly sitting on the skin is biting or not. Sandflies often gorge themselves without the bitten person feeling anything at all. The fact that the bite of the same sandfly a minute later on a different point may cause pain, suggests that only bites in the neighbourhood of nerve endings, or possibly special types of nerve endings, are felt.

First reaction.—In nearly all bitten persons there is no immediate visible reaction, except a tiny haemorrhage in the skin and perhaps a small droplet of blood oozing out of the wound. After the biting is completed and the pain ceases, no irritation or itching is recorded.

After an interval of usually 4 days or more there appear at the site of the bites raised, hard, reddish papules of 1 to 2 mm. in diameter, there is intense itching, generally at night, which subsides temporarily and starts again at intervals. The shortest observed incubation period for a first reaction was 3 days in one case; it was 4 days in five cases and in the other cases the first reaction appeared after 8 to 14 days, during which time no further biting experiments were carried out. In one case a reaction appeared as late as 34 days after the bite, during which time several biting experiments had been carried out. Judging from this and a number of other observations it seems that every individual bite reaction develops independently as regards incubation period and that, at least in the first few bitings, the process is definitely localised and not influenced by bites on other spots.

The incubation period seems to be longer in women than in men. In six out of seven female volunteers the first reaction appeared 8 to 14 days after the bite, while one gave no reaction at all to sandfly bites. All the cases with incubation periods of 3 to 4 days were males, and only in four out of sixteen males did the reaction appear later than 8 days after the bite.

There were only three instances out of thirty in which no first reaction appeared at all, and these cases subsequently proved absolutely refractory to

sandfly bites. Two of these three cases had not been previously exposed to sandfly bites.

In three cases white raised wheals with surrounding erythema appeared at the site of the bites within 5 to 15 minutes and disappeared in one hour without leaving any trace and without any subsequent reaction. Two of these cases had been bitten by sandflies 20 years ago in Mesopotamia, during the War, and one of the same two was also bitten 7 years ago by sandflies sent to London from Jerusalem. Their wheals were about 5 to 7 mm. in diameter.

One similar case (out of six) has been recorded by Boycott. His volunteer "H. D. W. was much exposed to phlebotomus in the East in 1917 to 1919. His bites began to wheal within 5 minutes."

In only one case, similar but smaller wheals, 3 mm. in diameter, were observed in a volunteer who had never left England. (It is perhaps interesting to note that this volunteer is the uniovular twin brother of Volunteer No. 1 who gave a papule reaction as described above and developed a wheal reaction only after 2 months continuous biting.)

In three cases the papules developed into small blisters. First the top of the papule became yellowish, fluid collected and blisters of about 3 mm. in diameter were formed. These dried up after several days and scaled.

Subsequent reactions.—In the later biting experiments on the same volunteers there was a considerable shortening of the incubation period as the following table shows.

TABLE I.
INCUBATION PERIOD IN DAYS.

Volunteer Number.	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6	Experiment 9
1	4	4	1	1	10 hours	10 min. wheals	
6	3	2	1	1	1	1	10 min. wheals
4	12	7	1	1	1	1	10 min. wheals
11	4	4	1	1	10 min. wheals		
13	1						
15	13	1					

The intensity of the reaction increased gradually in subsequent experiments, the papules were larger, more strongly inflamed and irritating. Inflammation of the surrounding area appeared, and became gradually more marked until in several cases the whole forearm became swollen and oedematous, the temperature raised, the lymphatic vessels to the elbow inflamed and the supra-trochlear glands enlarged. This occurred generally after the third or fifth biting experiment. In subsequent experiments on the same volunteers this general inflammatory reaction disappeared again gradually and in subsequent experiments the reaction became again similar to that of the first biting experiments, except that the incubation period remained brief. In still later experiments the character of the reaction changed completely and white wheals appeared immediately after the bite. This occurred in one case after six biting experiments during 1 month; in another case, after five experiments during 6 weeks; and in two cases after nine experiments during 2 months. During the whole time of the experiments the wheal reaction remained constant after it had once appeared, except that the wheals increased in size. They generally appeared 5 to 15 minutes after the bite and persisted for 30 minutes to one hour. Papules appeared in these cases as usual 24 to 48 hours after the bite and remained visible for 2 to 3 weeks, sometimes even 1 month. A brown pigmentation remained on the sites of the papules long after these had disappeared, so that all biting experiments were clearly mapped out on the skin during the 4 months in which the experiments were carried out.

A typical example of the whole course of the biting reactions in one volunteer is given in Table II.

REACTIVATION OF BITES.

An interesting phenomenon is the so-called repeating of the reaction as has already been described in the case of bites of other insects, *e.g.* bugs and lice, and is also a well-known phenomenon in other skin affections.

In several instances it was observed that old sandfly bites, which had given the papule reaction described above, were reactivated, after the original papules had disappeared, by new bites on a different place, *e.g.* on the other arm. The following cases are examples of this reactivation.

In Volunteer 1 the first bites on 14th November became inflamed again after 13 days (27th November) during which time three more biting experiments had been carried out. The reactivating bite on 27th November was accompanied by the strongest general inflammatory reaction observed in this particular volunteer.

In Volunteer 6, the first two sets of bites, on 8th and 11th December, flared up again on 15th December simultaneously with the bites of 14th December.

In Volunteer 11, the bites of 20th December and 12th January became inflamed again on 24th January together with the bites of 23rd January. (See Table II.)

TABLE II.
COURSE OF REACTIONS TO SANDFLY BITES IN VOLUNTEER 11.

Date.	Experiment 1.	Experiment 2.	Experiment 3.	Experiment 4.	Experiment 5.
20.12.34	12 bites. No immediate reaction				
24.12.34	Papules appear. No irritation				
7.1.35	Papules still persisting				
12.1.35	Papules going down	10 bites. No immediate reaction			
16.1.35	Papules disappeared. Pigmentation	Highly inflamed, itching papules appear			
20.1.35		Inflammation stopped. Papules small as in Experiment 1			
23.1.35		Papules gone down. No irritation	10 bites. No immediate reaction		
24.1.35	Papules inflamed again. Intense irritation	Papules inflamed again. Intense irritation	Papules much inflamed. Erythema, swelling of surrounding area. Intense irritation		
28.1.35	Papules smaller.	Less irritation	Irritation and inflammation less. Papules well defined, smaller	15 bites. No immediate reaction	
29.1.35				Highly swollen and inflamed area 7 × 7 cm. Lymphatics inflamed to elbow. Intense irritation	
30.1.35				Inflamed area larger, 15 × 8 cm. Oedema. Intense irritation	
4.2.35			Papules small. No irritation	Inflammation disappeared. Papules well defined. Less irritation	18 bites. Immediate wheal reaction
6.2.35					Inflammation and swelling 5 × 5 cm. All symptoms less marked than in Experiment 4
10.2.35					Inflammation disappeared. Papules well defined

Such a reactivation of old bites was observed only once in each volunteer during the course of the experiments.

DE-SENSITIZATION.

A complete de-sensitization was not observed during the relatively short period of the experiments, but the reduction in the intensity of the reaction, compared with that following the initial bites, in volunteers who had passed through a period of intense and generalised inflammatory reaction, may be considered as commencing de-sensitization.

Observations in Palestine indicate that, in time, a de-sensitization does take place, for people generally do not complain about sandfly bites except in the first few years of their residence, while those continuously exposed to sandfly bites for years finally cease to react at all, although originally they gave a strong reaction. Apparently the process of de-sensitization takes one or more years to be completed, the time required for de-sensitization varying in different individuals.

De-sensitization may disappear after the cessation of exposure to biting, *e.g.* in one case an exposure of several months to sandfly bites in Mesopotamia in 1917 was followed by de-sensitization from 1918 to 1920. No further exposure to sandfly bites occurred till 1924. In this year sandfly bites again gave a reaction followed by subsequent indifference from 1925 onwards.

LOCAL IMMUNITY.

In order to see whether a local immunity had been produced, sandflies were allowed to feed on persons who had developed a wheal reaction after continuous biting, on the sites where the original papule reaction had disappeared completely. The new bites were carried out 3 weeks or more after the first bites. The wheal reaction however developed on these spots in exactly the same way as on normal skin.

DISCUSSION.

Several authors call attention to the great individual differences in the reaction to sandfly bites. EYSELL (1924) describes a reaction (after DOERR) in which no pain is felt at the time of the bite, a small haemorrhage is produced and no further reaction appears. This probably corresponds to the ordinary papule reaction described above and because of the late appearance of the papules they have apparently not been connected with bites 4 to 14 days previously. He then describes an immediate wheal reaction with a subsequent papule reaction. These cases had probably been bitten previously. He also describes a third rather rarer kind of reaction, which starts with an enormous wheal followed by infiltration. 24 or 48 hours later a small blister which increases in size to 1 cm. in diameter appears on the site of the bite. Such a reaction is

generally accompanied by a generalised oedema of the regions concerned. EYSELL terms this a "hyperergic" reaction. This will be discussed later.

PAWLOWSKY, STEIN and PERFILJEW (1932) quote a number of descriptions of the reaction to sandfly bites by older authors which are in agreement with the observations recorded above. They give a photograph of the hand of a person bitten by *Phlebotomus major*, observed by Professor MARKOW in the Crimea, showing a blister reaction as described by EYSELL. In their own experiments they record the immediate appearance of wheals which is later followed by the development of papules which persist from 8 to 14 days. They do not state however with which species of *Phlebotomus* they were working and whether the people bitten in the experiments had been previously bitten or not. The observations recorded here all refer to *Phlebotomus papatasi* and it is of course possible that the reaction to the bites of other species is different.

In the light of the results described above it is necessary to correct several interpretations of the bite reactions given by BOYCOTT. He considers it as a rule that first bites do not give any reaction whatsoever. He states: "All volunteers were completely negative at the first biting." It seems however that he did not wait long enough for the first reaction to develop and that he carried out further biting experiments before the first reaction could have appeared normally. He then considered the appearance of the first reaction as a reactivation of the old bites by the later bites. He states: "It is a very striking thing to see a batch of bites on the left wrist remain completely quiescent and invisible for 10 days and then blaze out into irritable wheals some 20 hours after the same insects have been fed on the right arm."

A reactivation of old bites certainly does occur as recorded above but it can only be considered to have taken place in such cases in which a reaction had previously developed and subsided again. It appears therefore that there was no reactivation of old bites in BOYCOTT's cases but a normal late appearance of the first reaction.

In one of BOYCOTT's cases (Volunteer J. M.) a reaction appeared 16 days after the bite "after he had been rather freely bitten by 'midges' at Worth Matravers in Dorset." He assumes "that either *Phlebotomus* proteid sensitizes a human to the proteid of some English biting fly or that *Phlebotomus* itself occurs in Dorset." Since the above experiments show that the first reaction may appear as late as 14 days or more after the bite, there is no need for such an assumption and the case in question presumably showed a late first reaction without any connection with the later bites of other insects.

BOYCOTT uses the term "wheal" for a reaction which appears after 24 hours and which he describes as "singularly hard and lasting and a source of annoyance for a week or a fortnight." From this description it is safe to assume that the lesions referred to were what are termed papules in this paper except in the case which gave an immediate reaction and had been bitten during the War in the East.

The experiments recorded here confirm BOYCOTT's interpretation of the reaction to sandfly bites as an allergic phenomenon, *i.e.* a process of sensitization. The results obtained with phlebotomus are in accord with those observed by other authors for other insects, *e.g.* the experiments of KEMPER (1929) with bed bugs. In one case KEMPER obtained a shortening of the incubation period from 7 days at the beginning of the experiments to 3 hours after one year's continuous biting; and in another a shortening from 24 hours to an immediate reaction after 4 months biting. The wheals increased in size from 4.5 mm. in diameter at the beginning to 8.5 mm. after 2 months, and then became gradually smaller (6 mm. after 4 months, 3 mm. after 7 months, 2 mm. after 8 months) until after 9 months no reaction at all was obtained. The duration of the wheals gradually decreased from 9 hours in the beginning to a few minutes after 7 to 8 months. The state of complete lack of reaction was not permanent. After one month's interruption of the biting experiments new bites produced wheals of 3 mm. diameter.

The shortening of the incubation period in phlebotomus, the increase of the intensity of the reaction in subsequent bites until generalised inflammation appears, and finally the change in character of the delayed reaction with papules into an immediate reaction with transient wheals clearly suggest that a process of sensitization is taking place, which is then followed by a relatively slow process of de-sensitization.

It remains to be investigated whether the appearance of two different reactions, *viz.* papules and wheals, is due to different antibodies, or whether the wheal reaction represents a higher stage of intensity of the papule reaction. For the purpose of this paper the wheal reaction is considered as a sign of rather far progressed sensitization. The persistence of this state of sensitization throughout 20 years as observed in the three cases giving an initial wheal reaction is in accord with observations on other allergic phenomena. It seems that people in whom the process of sensitization is interrupted remain in this stage indefinitely.

The reactivation of old bites by later bites on different places is generally interpreted as a localized production of antibody by the first sensitizing bites, the minute traces of new antigen transported by the blood and lymph stream to these places being apparently sufficient to bring about the observed reaction (see HECHT, 1933).

The development of papules into blisters should probably not be considered as a higher stage of sensitization. In all cases in which the formation of blisters was observed, they appeared as a reaction to the first bites. No blisters ever appeared in people who reacted with papules at the beginning. It is possible that the formation of blisters is due to an especially marked individual disposition to exudative processes. Similar reactions with formation of big or small blisters have been reported by several authors for different insects, *e.g.* *Culicoides*, *Simulium*, mosquitoes, etc. The term of "hyperergic reaction" of DOERR for

the production of big blisters is therefore perhaps not very suitable and it remains to be investigated whether this "hyperergic" reaction is a stage of sensitization of the initial reaction with small blisters or whether it is the reaction of people constitutionally disposed to especially heavy exudation.

ATTEMPTED PASSIVE SENSITIZATION.

In order to get additional evidence for the interpretation as an allergic phenomenon, attempts to produce passive sensitization after the method of PRAUSSNITZ-KÜSTNER were carried out.

This method has first been used in the study of insect bites by HECHT (1930) in his attempts to establish the allergic character of the reaction to the bites of bugs and anopheles. He showed that a reaction with wheals could be transmitted passively by injection of serum intracutaneously into persons who had given no reaction previously. In the same paper HECHT gives an extensive review of the literature on the allergic character of the reaction to insect bites.

Experiment 1.—Blood was taken from Volunteer 7, who gave a strong immediate wheal reaction and 0.2 c.c. of his serum was injected intracutaneously into three persons. Of these, two (Volunteers 8 and 10) were completely refractory to phlebotomus bites and one (Volunteer 3) had acquired a complete non-susceptibility after having been bitten throughout many years by sandflies.

After 24 hours sandflies were fed on the site of the injection and on normal skin as a control.

In Volunteer 3, wheals of 4 to 5 mm. diameter surrounded by a strong erythema of 5 to 6 cm. diameter, accompanied by strong irritation, appeared at the site of the experiment within 5 minutes. The wheals and the erythema persisted for one hour. Faint reddish papules were still visible 24 hours afterwards. No reaction was observed on other places on which sandflies fed simultaneously.

There was a very faint and indefinite reaction in Volunteers 8 and 10.

Experiment 2.—Blood was taken from Volunteer 1 who had developed a wheal reaction after six biting experiments during one month. 0.2 c.c. of serum was injected intracutaneously into Volunteers 3, 8 and 19 (Volunteer 19 had developed an initial papule reaction after 5 days).

No definite positive reaction was obtained in Volunteers 3 and 19 but a very peculiar thing occurred in Volunteer 8. No positive reaction was obtained at the site of the injection, but when he was bitten again one hour later on a spot 10 cm. distant from the site of the injection a strong positive reaction was obtained with wheals and an erythema of 6 cm. diameter which persisted for 30 minutes. When he was bitten 3 hours later, 2 cm. distant from the place of the positive reaction, no reaction was obtained.

The positive result on Volunteer 3 cannot be considered as a plain Praussnitz-Küstner reaction as Volunteer 3 was in a state of de-sensitization and his allergic status cannot be defined exactly. No attempt is made to explain the peculiar reaction in Volunteer 8. The negative or doubtful results in the other volunteers also do not allow any definite conclusions as it is known that the Praussnitz-Küstner reaction gives negative results in a considerable percentage of cases. Altogether the experiments are too scanty for drawing conclusions and they will be continued.

THE REACTION TO SANDFLY BITES AND ITS RELATION TO THE "HARARA" OF PALESTINE.

Newcomers to Palestine often complain of a skin eruption of urticaria type. Face, arms and legs are covered with intensely irritating papules of different size. In some cases the papules develop into small blisters which in rare cases reach the size of 7 mm. in diameter. They are filled with clear or sometimes haemorrhagic sterile fluid. They usually become septic in a short time by scratching so that the original clinical picture is often completely obscured. This condition is commonly known as "harara" in Palestine.

DOSTROWSKY (1925) concluded, on the basis of clinical observation, that this skin condition was "nothing else than the reaction to phlebotomus bites" and he gives the following reasons:

1. *Season*.—Most cases of harara occur at a time when sandflies appear in Palestine, *i.e.* May to June.

2. *Localisation of Lesions*.—The papules of harara have exactly the same distribution on the body as sandfly bites.

3. Harara is mainly a disease of newcomers to the country; and after having excluded the effects of heat and solar radiation on clinical grounds, he assumes that the newcomers are exposed to the bites of an insect not occurring in their native country and he comes, *per exclusionem*, to the conclusion that this insect is *Phlebotomus* whose bites produce lesions similar to those observed in harara as described by DOERR and RUSS. The occurrence of haemorrhagic blisters in harara corresponds, according to DOSTROWSKY, to those described by EYSELL (1924) as a hyperergic reaction to sandfly bites.

SZENTKIRALYI and LOERINCZ (1933) in Hungary describe a number of rather typical cases of harara with papule and blister reaction and a few cases with generalised symptoms (lymphangitis, oedema, fever). They confirm DOSTROWSKY's observations and his views on harara as the reaction to phlebotomus bites.

Through the kindness of Dr. KATZENELLENBOGEN, Jerusalem, I had the opportunity of seeing a number of what he considered typical cases of harara. They corresponded in every detail with the lesions caused by the bites of phlebotomus as described above.

The lesions consisted generally of small reddish papules as described as typical in the third and fourth biting experiments at the beginning of the sensitization process. In some cases they were uniform, which suggests that they were just in the state of reactivation and, as this is generally accompanied by violent irritation, the patients usually go to the doctor at this stage. In other cases papules of different sizes and different stages of reduction were found. In a few cases blisters were present which were identical with those described above in three cases. No blisters of the "hyperergic" type of DOERR were seen and Dr. KATZENELLENBOGEN informs me that such cases are very rare indeed.

Several cases of harara with very distinct types of lesions were chosen and

single sandflies were allowed to bite on different points. The reaction to the bite of the sandfly was exactly identical in every case with the type of lesions already present.

Case 1.—Most of the lesions were papules of about 2 mm. diameter. A few lesions at the wrist were 7 to 8 mm. in diameter with surrounding erythema and very strong irritation. These were reported to be one day old.

Sandflies were biting at 5 p.m. Next morning papules of 2 to 3 mm. diameter appeared and grew during the day to the size of 7 mm. diameter with very severe irritation and surrounding erythema. After a further 24 hours they had returned to the size of the other papules. The experimental lesions corresponded in every detail to the ones already present. The occurrence of lesions of 7 mm. diameter as reaction to a single bite suggests that the patient was apparently at the stage of maximal inflammation and swelling as described above at the height of the sensitisation process.

Case 2.—Arms covered with blisters of about 3 mm. diameter filled with clear yellow fluid.

Sandflies were biting at 5 p.m. Next morning papules appeared at the site of the bites which developed within 24 hours into typical blisters corresponding in every detail to the blisters already present.

The following is a description of a case of harara of especially marked allergic character. I am indebted to Prof. A. KLOPSTOCK, Tel-Aviv for putting this report at my disposal.

The patient came to Palestine in the middle of July, 1933, and was bitten by sandflies in Jerusalem. In the end of July he felt that the papules produced by the bites became gradually larger and more strongly irritating. In the beginning of August in Haifa there developed a very annoying general condition, all papules (mainly on arms and legs) were strongly inflamed, old bites flared up and all were itching intolerably. The back of the hands became oedematous and asthmoid symptoms set in. All these symptoms disappeared after about 7 days. Since then the reaction to sandfly bites is not very strong and especially the subjective symptoms are much fainter than before. The patient in question has always been inclining to allergic reaction (hay fever).

The observations recorded above and the clinical observations suggest that the clinical condition known as harara is based on a certain allergic status, *i.e.* the height of the sensitization process. It is clear that in persons especially disposed to allergic reaction symptoms of harara are particularly severe and generalised.

It would therefore be more exact to define harara as the reaction to phlebotomus bites at the height of the process of sensitization.

SUMMARY.

1. The reaction to the bite of *Phlebotomus papatasi* has been studied in a population, in which previous contact with phlebotomus was definitely excluded.
2. The course of the reactions obtained confirms former views that a process of sensitization takes place in a relatively short time, which is later followed by a slow process of de-sensitization.
3. The view that the "harara" of Palestine is due to phlebotomus bites

is confirmed experimentally, and harara is defined as the reaction to phlebotomus bites at the height of the sensitization process.

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ORAL LEISHMANIASIS IN THE ANGLO-EGYPTIAN SUDAN.

BY

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Only one case of oral leishmaniasis has ever been reported from the African Continent—the one recorded by CHRISTOPHERSON (1914) in a Sudanese.

During the last eighteen months the writers have met with three cases—one British and two native—in the Anglo-Egyptian Sudan where in certain areas visceral leishmaniasis is fairly frequent.

Case I.—Native male, aet. 45 years.

A year ago he began to feel pain in all his teeth, especially the molars, and then the gums began to swell; later there was difficulty and pain on swallowing with the sensation of a foreign body in the throat. Examination showed a lobulated growth occupying the posterior part of the hard palate, the whole of the soft palate, the tonsils and extending along the inner side of the gums of the upper jaw (see Plate, Fig. 1). There were similar lobulated tumours in the nasopharynx and nares. The tumours were soft and spongy and in places showed ulceration. While scrapings from an ulcer on the tumour were negative, puncture of the growth revealed the presence of leishmania.

*We are greatly indebted to Dr. E. S. HORGAN for his pathological and bacteriological investigations and to Mr. N. MACDONALD for his excellent photographs.

We wish to thank The Director, Sudan Medical Service, for permission to publish this paper.

The liver was enlarged 3 in. below the costal margin and the spleen which was hard extended 1 in. below the level of the umbilicus. No leishmania were found by splenic puncture. Examination of the blood did not show any abnormality or any parasites. The Kahn reaction was negative.

Treatment.

This case was under observation for 5 months and during this period he was given intravenous injections of antimony tartrate (grains 14) followed by neostibosan (grains $32\frac{1}{2}$). Six weeks later another course of antimony tartrate injections totalling $22\frac{1}{2}$ grains was given.

At the end of the second course his condition had greatly improved; the tumours in the mouth, the nose and throat had markedly decreased and the patient could breathe easily and sleep undisturbed. There was little alteration in the size of the liver and spleen.

Four months after his discharge from Khartoum Hospital he was admitted to another hospital with ascites and emaciation and died shortly afterwards. Unfortunately no note was made of his oral condition nor was a postmortem examination carried out.

Case II.—British male, aet. 41 years.

1927.—Noticed purple patches on gums beside eyeteeth. Consulted dentist at home. Pyorrhoea diagnosed.

1927 to 1929.—Despite treatment, grew worse and gums swelled and pus formed.

1930.—Pipestem accidentally driven into palate and wound refused to heal. Had long sick leave: saw many specialists, surgical, dental, pathologist, etc. Diagnosed as chronic lupus, carcinoma atypical, etc.—later these diagnoses were withdrawn. All teeth extracted as condition considered of dental origin.

1930 to 1934.—Condition spread over palate and throat and ulcers present on gums, throat, floor of mouth and palate. Cheeks, tongue and lips swelled at intervals and partially subsided only to swell again. Unable to wear dentures with comfort and only able to wear them at all when swellings subsided a little.

1934.—Seen by one of us when floor of mouth, gums, tongue, cheeks and lips were swollen and apparently in state of chronic inflammation with small ulcers.

Direct smears from ulcers were negative but a smear from a freshly excised portion of gum showed leishmania. The floor of mouth, palate and gums, and also the inner aspect of the cheeks, had the appearance of granulation tissue with small ulcers. (This patient's personal servant had had kala-azar in 1927.)

Section of excised tissue showed a comparatively avascular granulation tissue rich in lymphocytes, large mononuclears, endothelial and plasma cells. No parasites were seen in section. The section might have been one of any subacute infection.



FIG. 1. Case I.

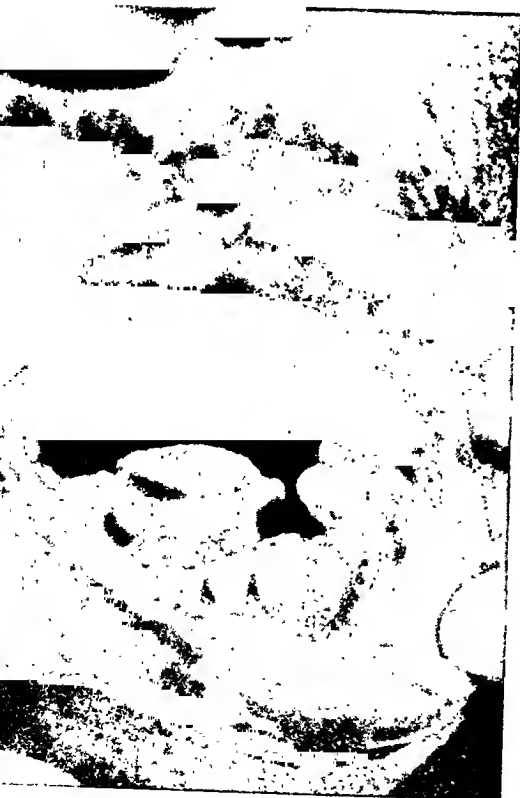


FIG. 2.

Case III. Before treatment.



FIG. 3.

Case III.

The lower incisor teeth instead of being loose were now firmly fixed. There was no alteration in the size of the liver and spleen nor had the pigmentation of the face and chest changed.

CONCLUSIONS.

(1) Three cases of oral leishmaniasis (espundia)—one British and two native—are reported and the results of treatment are given.

(2) Antimony is effective in curing this condition but must be given in larger doses and over a longer period than in visceral leishmaniasis; compare results of treatment of Case I with Cases II and III.

(3) Two of the three cases give a history of injury to the mouth, and trauma of the oral mucous membrane appears to predispose to local infection with leishmania.

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LYMPHOSTATIC VERRUCOSIS.*

BY

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LOEWENTHAL (1934) has described from Uganda eleven cases of an obscure foot lesion which he named "lymphostatic verrucosis." The disease differs from true "mossy foot," and is associated with chronic oedema and elephantiasis. The following case would appear to be an example of this condition.

The patient was an Ibo male farmer aged about 50, who was admitted to a district hospital complaining of septic condition of both feet, duration 1 year; and ankylosis of right knee, duration 9 months. There was no history of local injury.

Both feet were septic and very foetid. The plantar surface was moist and sodden, and was covered with innumerable, dense outgrowths (Plate, Figs. 1 and 2). Most of these were filiform in appearance, and varied in size from fine, hair-like processes to structures as thick as a stout needle. Some of the outgrowths were nodular, the largest being about the size of a large pea. Except for a few nodules on the dorsum, the outgrowths were confined to the plantar aspect and particularly the sides of the feet. This was unlike LOEWENTHAL'S cases, in which the "moss" did not appear on the plantar surface. On incision, the condition seemed to affect the skin only, the subcutaneous tissue being somewhat oedematous but otherwise normal. No fungus was seen in scrapings from the outgrowths, nor was there anything suggesting leishmania from any of the nodules. A septic condition of the toes and nails was present. There was no itchiness, no pain or tenderness, and there were no sinuses. The skin of the dorsum of the feet, and the skin of the legs was thickened and ichthyotic, but there was no pitting on pressure. There was fibrous ankylosis of the right knee, which was fixed in the semi-flexed position. There was slight oedema of the right thigh, and very slight, but definitely noticeable, elephantoid condition of the skin of the scrotum and penis. The spleen and liver were not palpably enlarged. Small, painlessly palpable femoral and inguinal glands were present.

There was no history of syphilis, but there was a doubtfully negative history of yaws.

Blood: Kahn strongly positive. No malaria parasites or microfilariae.

Stools: Ova of ancylostome and ascaris.

Urine: No albumin, casts, or sugar.

By the courtesy of Dr. ELMES, the Pathologist at Lagos, the following report was obtained on specimens forwarded for section:

"1. The largest piece. (i) a structure like new growth, which might be neuroma or angioma or neither. (ii) Hyperkeratosis. (iii) Great sub-epithelial oedema and telangiectasis."

"2. Sections from blocks show the hyperkeratosis, the myxomatous or oedematous condition, and the telangiectasis."

* Published by permission of the Director of Medical and Sanitary Service, Nigeria.

Treatment.—A course of iodides and N.A.B., with local antiseptic applications and rest of the patient in bed and elevation of the feet, produced only slight temporary diminution in the size of the verrucosities. The grossly septic and foetid condition of the feet made the application of an elastic binder for any prolonged period out of the question.

It was later ascertained that the patient died at home about 3 months after leaving hospital, *i.e.*, about 18 months after the onset of disease, presumably from toxæmia and exhaustion from septic absorption.

COMMENT.

Various diagnoses were offered by colleagues who saw this case or its photograph: "verrucose leishmaniasis"; "dermatitis verrucosa"; "mossy foot." The condition only superficially resembles the real "mossy foot," which is probably very rare in West Africa. "Mossy foot" is said by MANSON-BAHR (1931) to be caused by a fungus, and to occur usually only on the dorsum of the foot; the sole of the foot escapes, and the full development of the disease is slower than in the present case. The clinical and histological appearances agree essentially with those of the "lymphostatic verrucosis" of LOEWENTHAL, though the local distribution is somewhat different. He recognises three stages of the disease, namely, a preliminary "velvet skin" stage, a verrucose, and an ulcerative septic stage. As in his cases, the essential pathology here would appear to be one of lymph stasis, oedema, and hyperkeratosis. The slight but definitely elephantoid appearance of the thigh and genitalia in the present case suggests that the condition is a manifestation of, or is at least associated with, elephantiasis, which might or might not have been due to filariasis, an endemic disease in these regions. The undoubted association of the disease with elephantiasis inclines one to venture the opinion that the name "elephantiasis verrucosa" is probably just as good a one for it as that proposed by LOEWENTHAL.

SUMMARY.

1. An example of the condition termed "lymphostatic verrucosis" has been described.
2. Its essential pathology, namely, lymph stasis, chronic oedema, and hyperkeratosis, is stressed.
3. It is suggested that, having regard to its association with elephantiasis, an alternative name, namely, "elephantiasis verrucosa," might also be applied to the condition.

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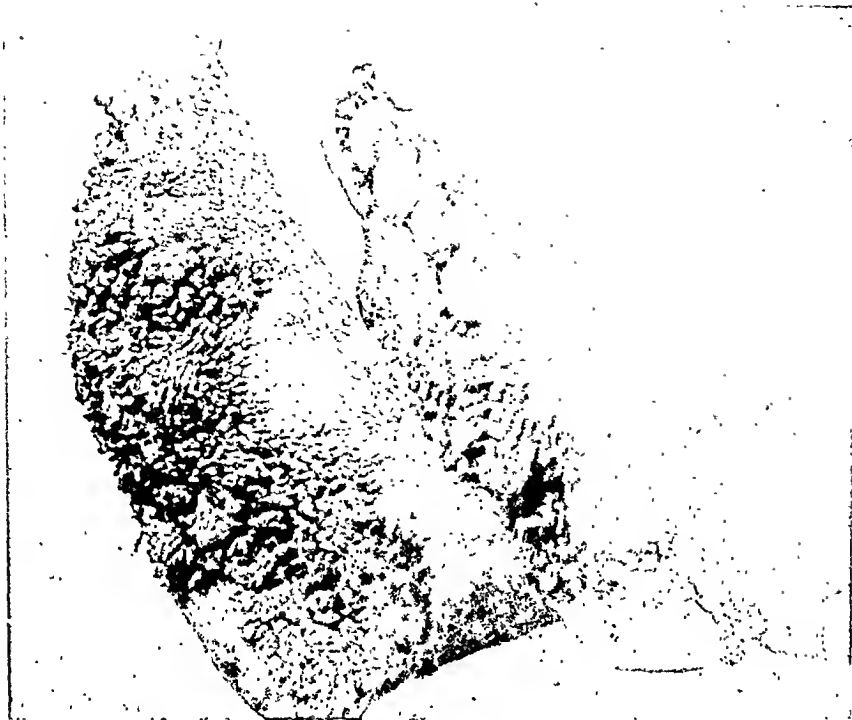


FIG. I. Plantar aspect of feet.



FIG. II. Dorsum of feet.

FURTHER STUDIES ON THE POLYNUCLEAR COUNT IN IRAQ.*

BY

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In a recent study by one of us (KENNEDY, 1935) it was reported that the polynuclear count, or COOKE and PONDER's modification of the Arneth count, of the natives of Iraq showed a marked shift to the left as compared with the health standard in Britain. This would at first indicate an infective state. The men studied, however, were all in apparent health; and as the limited facilities available prevented a full clinical investigation, one could not exclude the possibility of widespread yet low-grade infection (helminthic, malarial, gonorrhoeal, and so on). Still one was unwilling to accept on the evidence that so widespread and active an infection did exist among the subjects. The possibility of a racial factor entering in had also to be considered as the material was drawn from Kurds, Arabs and Jews. To discover whether infection or race were influential, further counts were made on 134 British airmen and officers stationed at Hinaidi near Baghdad.

*This paper is published with the permission of the Director-General of Health, Baghdad, and of the Air Ministry.

This population afforded considerable advantages. The medical standard for admission to the Royal Air Force is a very high one. All the subjects had good health records since coming East. None had shown minor ailments (colds, sore throats, diarrhoea) for over three weeks. Many had just undergone the rigorous annual medical examination of the R.A.F. pilot. In fine, the health standard of the group is comparable with the stringent selection of COOKE and PONDER's original series. Most of the men had been in Iraq for over a year, and all for at least six months. The environmental conditions were very uniform throughout the group, the age distribution was narrow. In this way we obtained a population which gave a very fair contrast to an indigenous "healthy" sample taken at random. Additional counts were made of 13 healthy Arabs to bring the original figure of 121 to the number of the British group.

The samples were collected between May and June, that is about a month earlier than the previous Iraq series, but the temperature was abnormally high for the season, the shade maxima varying between 100° and 115° F. This was the same range as on earlier occasion. The smears were all made between 10.00 hours and 12.00 hours. They were stained with standard Giemsa, and 100 cells examined. Both observers counted each slide to eliminate personal errors, and the different figures thus obtained agreed very closely. As the methods and criteria of the count were detailed in the earlier paper in this journal (KENNEDY, 1935) and in COOKE and PONDER's book, further reference to them is unnecessary here.

A group of representative pathological cases, British and Iraqi, were also studied to discover the extent of the shift in disease. Since intensity of illness is an incommensurable, no "accurate" comparison can be made with a like group in Britain. However, the clinical picture of acute pneumonia, subacute salpingitis, and so on, is sufficiently defined to give a permissible degree of comparison. The full counts of a representative selection of these cases is given in Table II (page 296).

When the research was partly completed the results were found to agree closely with the Iraqi figures, and this suggested that animal counts might also be compared with British standards. Rabbits were chosen as KENNEDY (1931) had already published figures for them. Owing to scarcity only 32 rabbits were used. Postmortem examination showed that they were healthy.

RESULTS.

The weighted mean of the polynuclear count is a more sensitive measure than the Arneth index. It is found by multiplying the total cells in each class by the number of the class, summing the results, and dividing by the number of cells counted. The average weighted means and relative statistics are given in Table I, in which the normal figures for Iraq, Britain (COOKE and PONDER), and KENNEDY's figures have been included for comparison, and in addition pathological groups for Britain and the East.

TABLE I.

	Number of Cases.	Average Weighted Mean.	Standard Error.	Standard Deviation.	Coefficient of Variation.	High Mean.	Low Mean.
Iraq	134	1.994	0.024	0.273	13.7	2.68	1.37
Hinaidi	134	1.935	0.019	0.222	11.42	2.50	1.35
Edinburgh	90	2.628	0.020	0.190	7.23	3.05	2.18
Britain (Cooke and Ponder)	90	2.740	0.019	0.180	6.57	3.11	2.47
Pathological (Eastern)	51	1.380	0.019	0.164	11.9	1.87	1.08
Pathological (British)	78	1.930	0.031	0.277	14.42	2.45	1.32

The average weighted means for the airmen, and for the native Iraqi after adjustment for the additional cases, corresponded to counts which actually occurred as follows :—

	I	II	III	IV	V	Weighted Means.
Airmen	30	49	18	3	0	1.94
Iraqi	26	51	21	2	0	1.99
„	36	34	25	5	0	1.99

The presence of double maxima indicates an unsteady state of polymorph production, but none such were observed in either the present or the earlier Iraqi series.

Applying the formula for standard deviation of the difference between two means to the Hinaidi and Iraq results, it is found that $\sigma \text{ diff.} = 0.0290$ while the difference between the means is 0.059. This is only very little more than twice the former figure which indicates that the difference in the distribution is not statistically significant.

PAI has recently published some results of the polynuclear count of Chinese and British in Moukden (China) and British in Leeds. His figures may be considered with the present ones as his counts have been personally checked by COOKE, who has likewise controlled the counts made by one of us. MACLEOD has conducted a similar study, and we can also consider his results along with our own, as he too acquired the technique from the same group of workers. It has not been thought justifiable to discuss at any length the results of other investigators who either used ARNETH'S original criteria, or have not defined exactly the basis of classification of the cells.

Figure 1 gives frequency polygons of the weighted means of the airmen at Hinaidi, the amended Iraqi group, and COOKE and PONDER's original health standard. While the broad facts of a population distribution are well enough shown by such a diagram some of the finer points are obscured. A closer comparison can be made by using an ogive curve which records every observation. This is given in Figure 2 for KENNEDY's British results, PAI's healthy Chinese, the native Iraqi, and the airmen. The difference between the first two and the last two is obvious, and equally striking is the similarity between the British airmen stationed in Iraq and the indigenous population. As infection

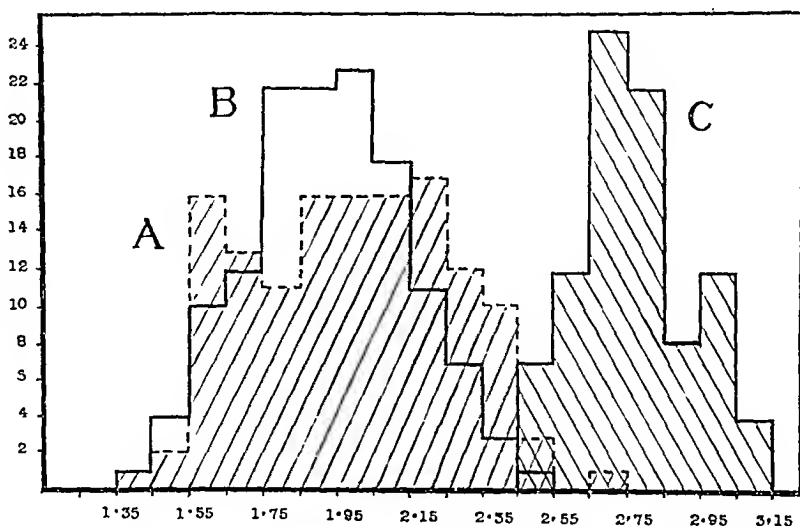


FIG. 1.—Frequency polygons of weighted means : A, British airmen : B, Iraqi nationals : C, COOKE & PONDER's health standard group.

has been eliminated, and as diet cannot be considered a potent influence since the airmen's food does not greatly differ from that at home, it may be concluded that race is not a significant factor. The remaining possibility is some environmental influence, and climate appears at once to be the most obvious one. The importance of climatic conditions in relation to the polynuclear count has already been indicated by KENNEDY and FLINT (1930) who found that cases of surgical tuberculosis treated in the Swiss Alps had a more right-handed count than similar cases in Britain. This was then ascribed to heliotherapy as KENNEDY and THOMPSON (1927) had shown that irradiation with ultra-violet light produced a deflection of the counts in rabbits.

MACLEOD studies 25 cases from each of 11 widely scattered localities. He does not give extended counts, but for comparison we have incorporated the means and extreme ranges of his figures in the graph, Figure 3. ABELS's results

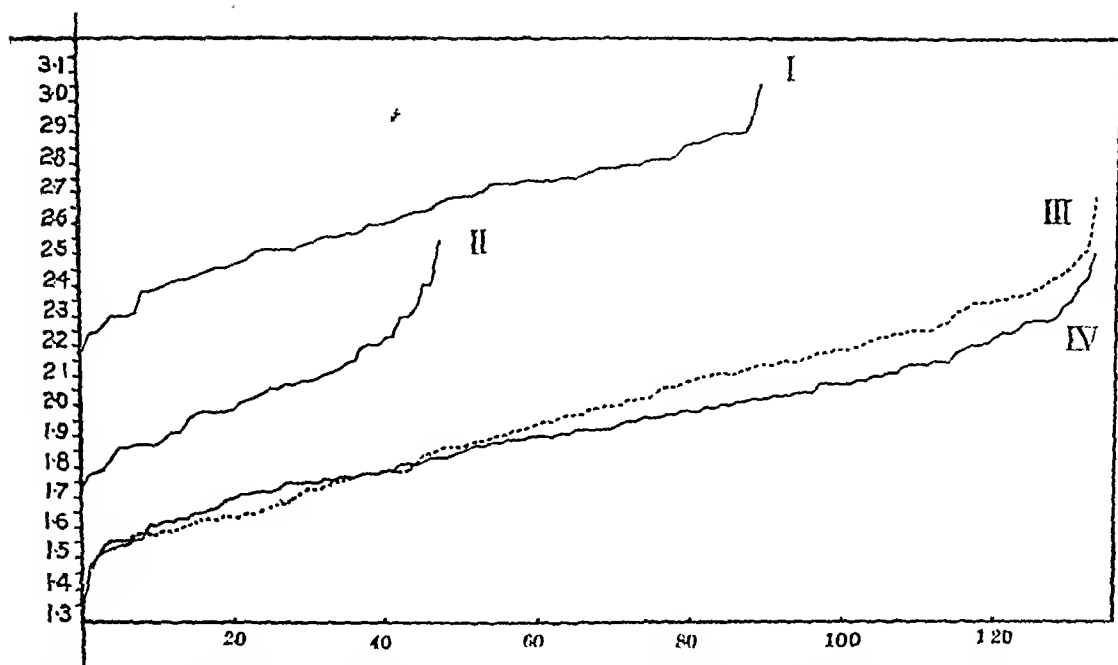


FIG. 2.—Ogive graphs of weighted means: I. KENNEDY's Edinburgh group; II, PAI's Chinese group; III, Iraqi nationals; and IV, Airmen at Hinaidi.

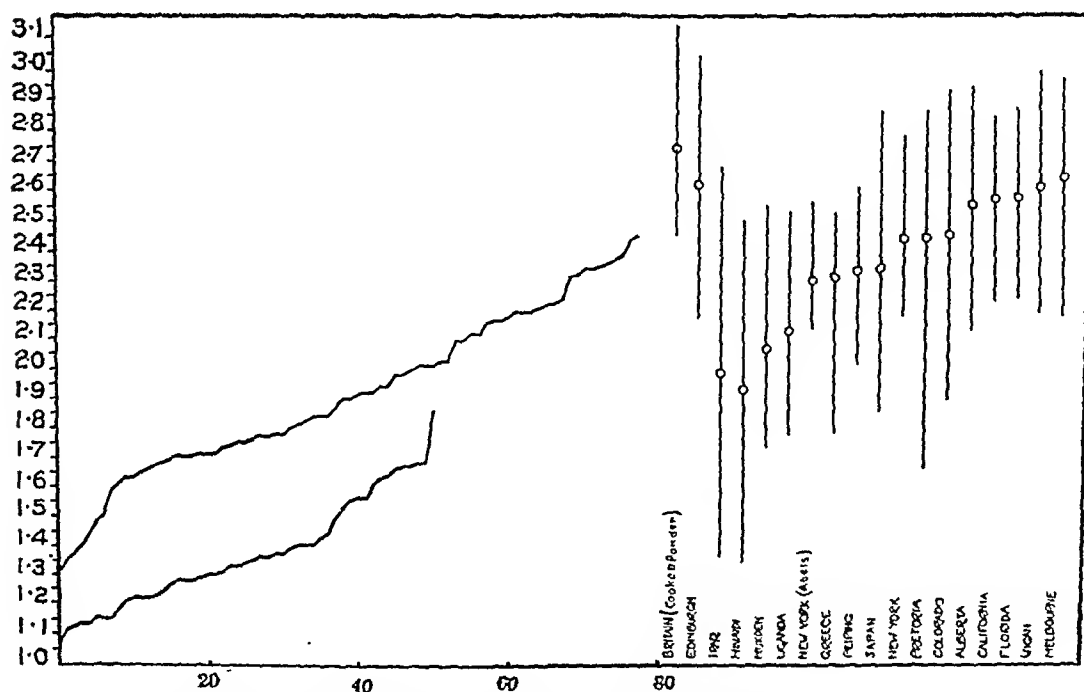


FIG. 3.—Ogive graphs of weighted means of British pathological cases, upper curve; and Eastern pathological cases, lower curve. Line diagrams of the ranges and average weighted means of the present data (healthy cases), and that of authors mentioned in the text. The diagrams from Greece to Melbourne inclusive are derived from MACLEOD.

r New York are included, and LYNDHURST DUKE's for Uganda. The weighted means for the latter have been calculated from the author's tables, and inserted because of the interest of the locality ; but they cannot be considered strictly comparable as the original ARNETH criteria were used in this case.

To economise space Figure 3 also contains ogive curves for pathological groups, one British, one Eastern. The British curve is a composite one consisting of means calculated from the counts given by COOKE and PONDER (45 cases) by PAI (13 cases from Leeds) and 20 of our own cases from Edinburgh (unpublished). The counting standards of all these three are comparable. The cases included are pleurisy, pneumonia, osteomyelitis, puerperal sepsis, and the like. The group we have called Eastern is composed of 41 of our own cases, British and Iraqi, to which we have added 10 calculated from PAI's figures, as they are spaced evenly through our own distribution, and as we are only interested here in showing that a significantly greater left hand shift occurs with pathological cases in the East than in Britain. Extended counts of 21 of our cases are given in Table II to illustrate the polynuclear picture.

TABLE II.

	Classes.					Weighted Means.
	I.	II.	III.	IV.	V.	
Malaria	92	8	0	0	0	1.08
Bilharziasis	89	10	1	0	0	1.12
Phlebotomus fever	88	11	1	0	0	1.13
Malaria	84	15	1	0	0	1.17
Lymphatic leukaemia	82	17	1	0	0	1.19
Puerperal sepsis	79	20	1	0	0	1.22
Snake bite	80	17	3	0	0	1.23
Pleurisy & effusion	77	20	3	0	0	1.26
Salpingitis	75	21	4	0	0	1.29
Acute rheumatism	74	23	3	0	0	1.29
Splenic anaemia	73	24	3	0	0	1.30
Septicaemia	76	18	6	0	0	1.30
Malaria	74	20	6	0	0	1.32
Pleurisy	69	26	5	0	0	1.36
Acute appendicitis	69	25	6	0	0	1.37
Pneumonia unresolved	64	32	4	0	0	1.40
Osteomyelitis	58	34	7	1	0	1.51
Burns & sepsis	56	34	10	0	0	1.54
Pyelitis	53	38	9	0	0	1.56
Phlebotomus fever	53	39	7	1	0	1.56
Parametritis	50	38	10	2	0	1.64

Only 32 rabbits were available while the group determined in Britain numbered 73, but when the weighted means of each were plotted as frequency polygons, the Iraq rabbit counts are plainly much more left handed. If the numbers could be equalised there is no possibility of the graphs approximating closely enough to obliterate the difference.

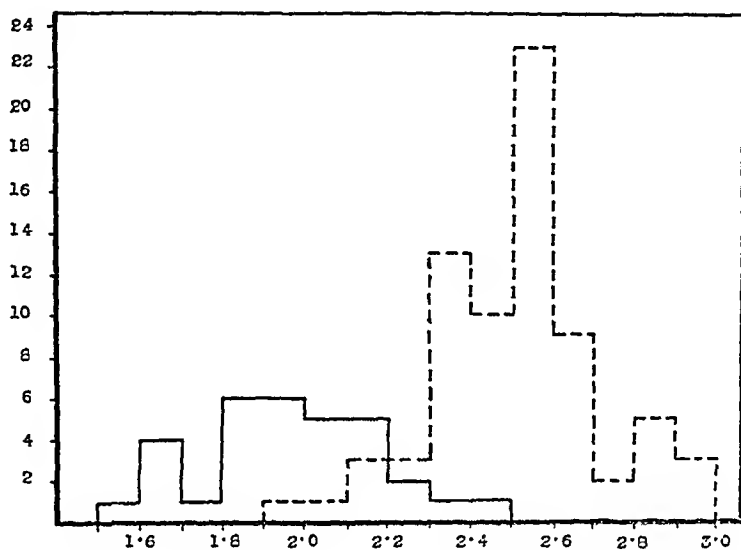


FIG. 4.—Frequency polygons for weighted means for rabbits in Iraq, whole line ; and in Britain, dotted line.

DISCUSSION.

At the present stage little can be offered as an explanation of the marked differences in the polynuclear counts detailed above. Climatic conditions appear to be a likely determinant, the ultra-violet radiation and heat are probably important factors. Both are certainly capable of causing a left-handed deviation of the count. The former has been referred to already, and one of us has found that exposure to the intense dry heat of a Turkish bath causes a significant left-hand shift (KENNEDY, unpublished observations).

Left-hand deviations are produced by stimuli to the leucogenic centres, by accelerated removal of polymorphs from the circulation, or by both. Isolated stimuli to the bone marrow, *e.g.* ether anaesthesia, produce an unsteady state of the count, that is, successive counts show the wave of increase passing from Class I through Class II to Class III and so on, frequently emphasised by the presence of double maxima. The effect of a single stimulus dies away in about three weeks. Continued stimulation without a parallel acceleration of removals would produce a leucocytosis, the reverse of leucopenia. We have no indication that any of these occur in our healthy populations. Here it would appear that

a balance has been attained between increased removal and leucogenesis. There is some evidence indicating the probability that the rate of leucocyte destruction and removal from the circulation exercises a kind of automatic control on cell production, the stimulus being derived from the breakdown products of the polymorphs. The reason for the shorter life of the leucocyte in the circulation still requires elucidation.

Surprisingly little interest has been taken in the relation between the physiology of the normal individual and meteorological conditions. This is especially striking when one reviews the vast literature on the seasonal incidence of disease, and on the prevalence of certain diseases, non-parasitic or unconnected with insect vectors, in definite climatic regions. There is much need for systematic exploration of the field, for example, along the lines of PETERSEN's illuminating and stimulating researches. It is to be hoped that further opportunities may be found here and elsewhere for more extensive studies of a like nature.

SUMMARY.

The polynuclear count of healthy British airmen in Iraq is not significantly different from that of the indigenous population. Both these groups are definitely deviated to the left as compared with the health standards in Britain. Pathological cases in Iraq show a greater degree of deviation than similar British cases. Normal rabbit counts are also more left handed. It is suggested that the most probable causative factor is climatic.

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THE DEMONSTRATION OF THE HAEMOPOIETIC PRINCIPLE IN CHRONIC PELLAGRIC ACHYLIA.

BY

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In a series of investigations on anaemias in Egypt, the extreme rarity of Addisonian anaemia was demonstrated (SALAH, 1935), despite the presence of various conditions associated with achylia gastrica.

Pellagra is of common occurrence in the farmers; the presence of achylia gastrica was demonstrated by the writer, using the alcohol, histamine method, in one third of these cases. This is a far smaller figure than that reported by other workers, and previously by the writer, using the fractional oatmeal method. Investigations into the nature of this achylia in pellagra suggest an inflammatory basis which progresses with recurrent attacks to an atrophic condition of the gastric mucosa with a tendency to recovery of gastric secretion in exceptional cases only (SALAH, 1933).

The great similarity between pellagra and Addisonian anaemia shows itself not only in the clinical manifestations common to both conditions, *e.g.*, glossitis, achylia gastrica, cord lesions, remissions, etc., but also in their etiological origin from a deficiency state. Their response to various kinds of treatment is also, more or less, similar. Vitamin G deficiency in rats could be prevented and cured by ventriculin (SPIES and GRANT, 1933). Liver diet and extract have shown beneficial effects in certain manifestations of pellagra in the hands of some workers (RUFFIN and SMITH, 1934), although this was not the experience of the writer when misleading temporary improvements resulting merely from the hospitalization were excluded. Yeast and marmite possess a prompt curative effect on some pellagric manifestations, and have been shown to produce a remission in some forms of pernicious anaemia, *e.g.*, tropical megalocytic anaemia of India (WILLS, 1931) and some cases of hyperchromic anaemia associated with idiopathic steatorrhoea (VAUGHAN, 1932).

That acquired achylia could give rise to a megalocytic hyperchromic anaemia is accepted; this should only occur if the process in the stomach affects as well the formation of the intrinsic haemopoietic principle by the gastric mucosa.

Thus, demonstration of the intrinsic haemopoietic principle in the gastric secretion of chronic pellagic patients with achylia gastrica is attempted in this paper to explain the non-occurrence of a pernicious blood picture in these cases.

CASTLE's (1929) test for demonstrating the haemopoietic principle in any gastric secretion by mixing it with beef and introducing it into the stomach of a P.A. (pernicious anaemia) patient and examining his blood for a reticulocytic crisis could not be applied in Egypt owing to the absence of the test patient (a pernicious anaemia patient). MORRIS (1933) has shown that if a concentrated normal gastric juice, is injected intramuscularly into a P.A. patient, a prompt and sustained reticulocytic crisis and remission follows. This has been recently confirmed by TOCHOWICZ (1934). So, a modification of CASTLE's test will be the injection of the concentrated gastric juice to be tested into a P.A. patient instead of incubating it with beef and then introducing it orally.

In the present investigation, search was made for an experimental animal with anaemia responding to the injection of normal gastric juice by reticulocytic crisis. Pigeons on grain diet were shown to develop megalocytic anaemia, and it was suggested that these might be used for testing the efficacy of various liver extract preparations by noticing the reticulocyte response following their administration. VAUGHAN (1930), however, showed the great lability of the reticulocytes of pigeons and thus their unsuitability for such a procedure; the writer obtained similar results.

Recently SINGER (1935), using white rats, was able to get their reticulocytes down to a constant level by putting them on a milk diet; if now normal gastric juice is injected into these rats, a reticulocytic rise occurs within 2 or 3 days and disappears on the 5th day. Using this method, R.R.R. (rat reticulocytic reaction), he was able, in controlled experiments, to show that this reaction is positive with gastric secretion from normal stomach, achylic stomach without anaemia, achylic hypochromic anaemia, haemolytic anaemias; and it is negative if gastric secretion of Addisonian anaemia is used or if the normal gastric juice is heated to destroy the intrinsic principle. Accordingly he concluded that the reaction demonstrates the presence or absence of the haemopoietic principle in any gastric secretion.

These results have been later confirmed by HITZENBERGER and PASCHKIS. BARATH and FÜLÖP (1935) have utilized this reaction for demonstrating even quantitative differences in the content of haemopoietic principle.

Following SINGER's technique, 22 white rats were put on milk diet and bread; after 2 to 4 weeks, the reticulocytes fell to the level of 4-19 per mille and remained at that level after an initial value of 80-100 per mille. The reticulocytes were counted by a method previously described by the writer (SALAH, 1933), which was used instead of KAMMERER's method employed by SINGER, as it showed more constant results.

Five chronic pellagra patients with achylia gastrica negative to histamine

and two ankylostome anaemia patients with achylia gastrica (one negative and one positive after histamine) were chosen for this purpose.

The gastric juice was collected after histamine injection of 1 mg., the fasting stomach being repeatedly washed with distilled water beforehand.

The following table shows the results obtained by injecting the gastric secretions of these cases into white rats with constant low reticulocytic counts ; each was injected into two rats. In addition, the results of injecting the gastric juice of three of these cases after the destruction of the haemopoietic factor by heat, are also given for comparison.

Case.	Condition of Gastric Secretion.	Reticulocytes of Rats.					
		Before Injection		After Injection of Gastric Juice, 5 c.c.		Before Injection.	After Injection of <i>Heated</i> Gastric Juice, 5 c.c.
		Rat 1.	Rat 2.	Rat 1.	Rat 2.		
1 Chronic pellagra, S.C.D.	Achylia : histamine negative } do.	14	9	38	29	15	11
2 Chronic pellagra, Lateral sclerosis		12	16	42	38	14	14
3 Chronic pellagra, Bilharzial dysentery		16	13	45	46	—	—
4 Chronic pellagra	do.	14	15	52	32	—	—
5 Do.	do.	12	10	36	53	11	13
6 Ankylostome anaemia	do.	18	9	57	33	—	—
7 Do.	Achylia : histamine positive }	13	19	43	47	—	—

It can be seen from the above table that the achylic gastric juices of five chronic pellagra patients and two ankylostome anaemia patients are capable of producing reticulocytosis in rats kept anaemic with low reticulocytes on milk diet, *i.e.*, a positive R.R.R. (rat reticulocytic reaction). According to SINGER this means the presence of the haemopoietic principle in the gastric secretion of these cases.

These findings show that the gastric lesions of chronic pellagra do not interfere with the formation of the intrinsic haemopoietic principle ; thus explaining the non-occurrence of hyperchromic megalocytic anaemia in this condition.

The donor was anaesthetized with 15 c.c. of 5 per cent. nembutal intra-peritoneally. The thoracic and cervical ducts were isolated and cannulated. The flow of lymph was free and very full of fat. The cervical lymph had a count of 1,720 microfilariae per c.c. and that obtained from the thoracic duct, 1,160 per c.c. The dog was bled to death at 12 o'clock noon from the femoral artery. A total of 1,050 c.c. blood was collected in beakers containing heparin dissolved in saline. The beaker was agitated continually during the bleeding.

The recipient was likewise anaesthetized with 8.5 c.c. nembutal. The thoracic and cervical ducts and a collecting lymphatic of the right hind leg were isolated and cannulated. A few granules of heparin were added to each cannula to prevent coagulation. The flow of lymph from the thoracic duct was free but slight massage was necessary to produce adequate flow from the leg and cervical ducts. A total of 450 c.c. blood was withdrawn from the right femoral vein and was replaced with 1,000 c.c. of the donor's heparinized blood injected into the same vein.

Ten minutes after completion of the transfusion the blood of the recipient showed 178,000 microfilariae per c.c. Approximately 0.5 c.c. of lymph taken at this time from the thoracic duct showed three active microfilariae. This lymph was slightly blood-tinged. Within the following hour 48 c.c. lymph was collected from the thoracic duct. The specimen was centrifuged at high speed. The sediment contained 18,200 microfilariae.

Twenty-five minutes after completion of the transfusion one microfilaria was found in 5 c.c. lymph massaged from the leg. A total of 211 microfilariae were obtained an hour later in 1.5 c.c. lymph from the leg. Samples of lymph (5 c.c. in all) taken from the cervical duct were negative up to 3 hours after transfusion. At that time, however, 9 microfilariae were found in 3.1 c.c. lymph.

Upon cisternal puncture 7 c.c. of clear fluid was obtained. This fluid was centrifuged at high speed for 5 minutes. The sediment contained 464 microfilariae. All microfilariae recovered from the recipient were alive and appeared normally active.

The dog was then killed by an intravenous injection of chloroform and autopsied. No abnormalities were noted. The tissues removed for later microscopic study were fixed in Zenker's solution.

Histology.

Histological study of sections of tissue removed and fixed at autopsy of both donor and recipient showed that the microfilariae are disseminated in every part of the body. They appeared in greatest numbers in the lungs, liver and kidneys. No larvae were found in sections of the stomach or intestines. So strikingly similar were the sections of both donor and recipient that they need not be considered separately.

The lung sections showed numerous larvae in the large arteries, veins and capillaries and an occasional one in the alveoli. There were in the liver numerous microfilariae extended in the veins and in the sinusoids.

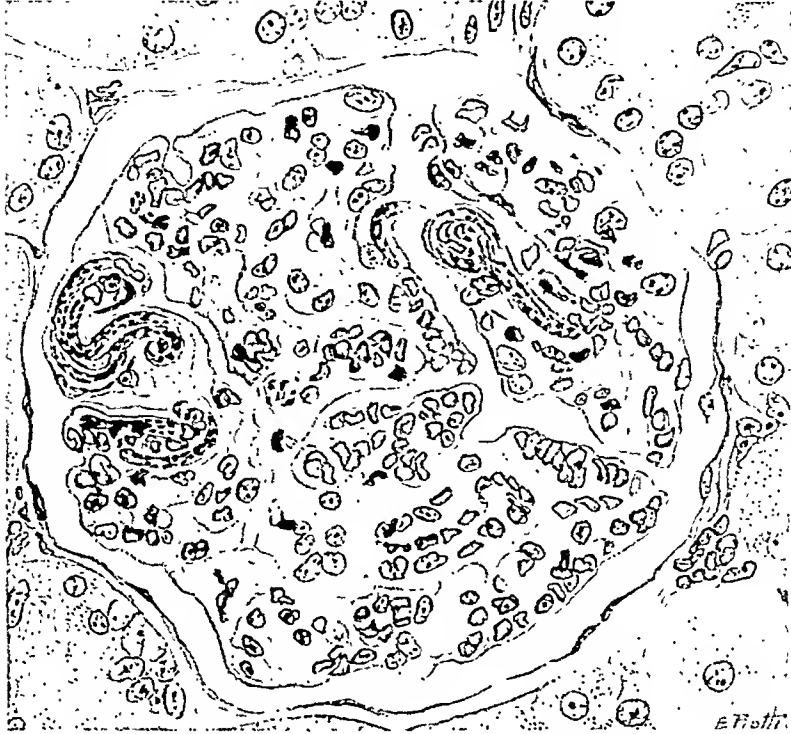


FIG. 1.—Microfilariae (*Dirofilaria*) in the capillaries of a glomerulus.

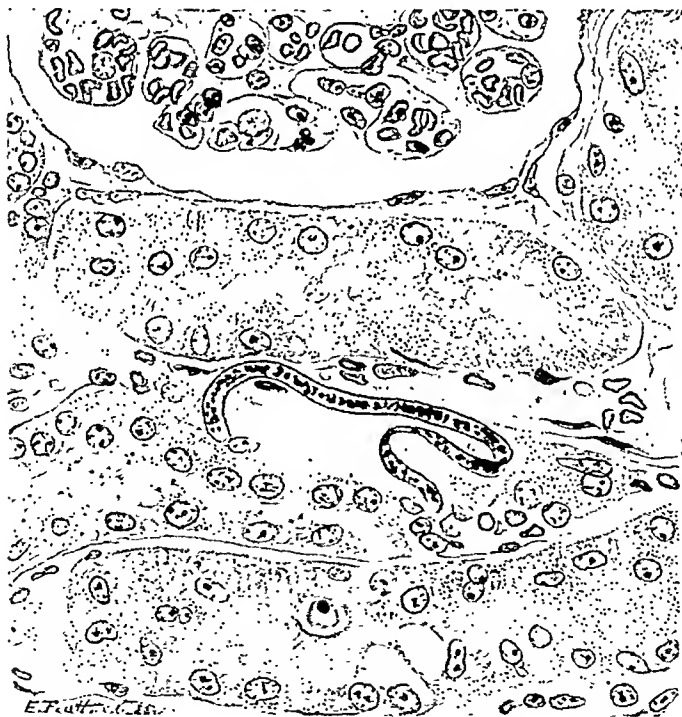


FIG. 2.—Microfilaria lying between tubular capillaries of the kidney. Whether this organism lives in or out of the intertubular capillary could not be determined.

The donor was anaesthetized with 15 c.c. of 5 per cent. nembutal intraperitoneally. The thoracic and cervical ducts were isolated and cannulated. The flow of lymph was free and very full of fat. The cervical lymph had a count of 1,720 microfilariae per c.c. and that obtained from the thoracic duct, 1,160 per c.c. The dog was bled to death at 12 o'clock noon from the femoral artery. A total of 1,050 c.c. blood was collected in beakers containing heparin dissolved in saline. The beaker was agitated continually during the bleeding.

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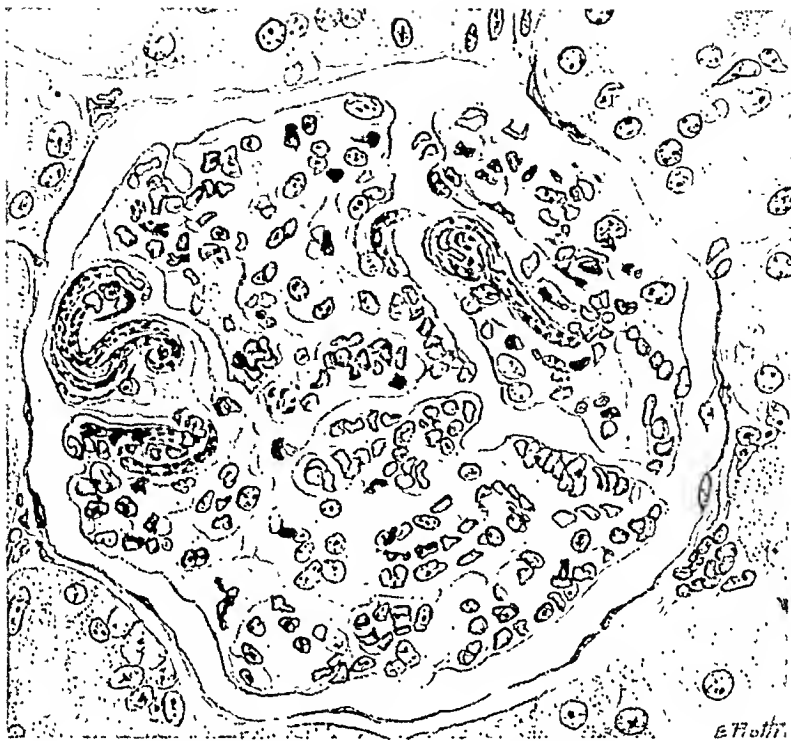


FIG. 1.—Microfilariae (*Dirofilaria*) in the capillaries of a glomerulus.



FIG. 2.—Microfilaria lying between tubular capillaries of the kidney. Whether this organism lives in or out of the intertubular capillary could not be determined.

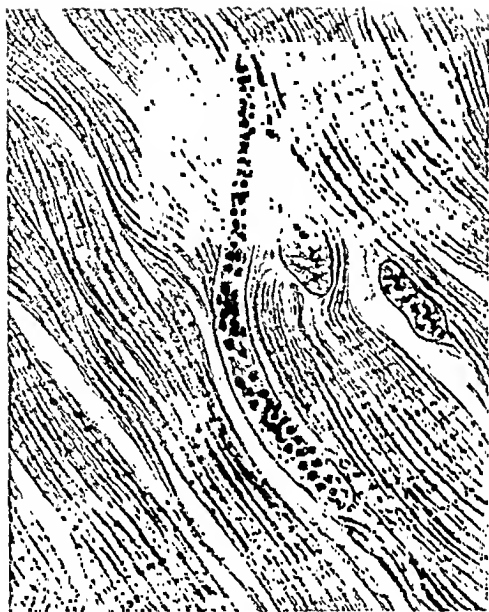


FIG. 3.—Microfilaria lying between muscle fibres of the left ventricle.

Note complete absence of a host reaction.

In the kidney microfilariae were frequently found in the glomeruli and in the intertubular capillaries. A glomerulus may contain from four to five organisms. When thus crowded with microfilariae the capillaries of the glomerulus occasionally showed some oedema but no other pathological change. The microfilariae appear, for the most part, to follow the course of the capillaries through a glomerulus and were much coiled. However, an occasional microfilaria was found extending straight across a glomerulus which would indicate that a few break out of these vessels at this point. It would appear, however, that very few are discharged into the urine, for no living or dead microfilariae were encountered in the sediment of about 40 c.c. urine withdrawn from the bladder of a heavily infected dog in a previous experiment. Although microfilariae may be held up in the glomeruli, it appears that the delay is temporary only, for fairly large numbers of larvae were found in the veins.

A few microfilariae were found about the capsule of the spleen and frequently occurred in the pulp of both donor and recipient. In an early experiment none was found in this organ of an infected dog.

The number of microfilariae encountered in lymph nodes was always small. They were found most frequently in the intermediary sinuses in the depths of the gland. The popliteal and iliac nodes and nodes from the mesenteries and anterior mediastinum were studied.

Tremendous numbers of microfilariae were noted in the large arteries of the pericardium. Numerous larvae were also found in the myocardium, particularly in the left ventricle. For the most part, they lay parallel to the muscle fibres but in several instances evidence of the capillary is not apparent and they appear to lie free between the muscle fibres.

Sections of brain tissue adjacent to the lateral ventricles showed numerous microfilariae in the veins and capillaries. None was seen in the brain tissue itself. In a previous experiment, however, a single larva was seen for nearly its entire length in this tissue. There was no evidence of the capillary about it nor in its immediate vicinity.

No gross pathological changes were noted at autopsy of either donor or recipient. The sections showed only an occasional slight oedema of the capillaries of a crowded glomerulus. All microfilariae noted appeared structurally normal and, for the most part, appeared to have been in active progression at the time of fixation. No cellular reaction of any sort has been noted against these parasites in this and previous experiments on the migration of microfilariae in the body of the host.

Fifty-two female and 39 male adult *Dirofilaria* were obtained from the right ventricle and pulmonary artery of the donor. None was found elsewhere. An examination of the reproductive system of 35 female worms showed all to contain undeveloped eggs, embryonated eggs with active embryos in various stages of development, and myriads of microfilariae.

Figs. 1 and 2 are drawn from sections of the left kidney and Fig. 3 from the muscles of the left ventricle of an infected dog of an earlier experiment. The relation of the microfilariae to host tissue shown in these drawings is typical

of that encountered in all the tissues of both the donor and recipient of the present study. No cellular reaction on the part of the host has been noted. The entire absence of a foreign body reaction is striking. The fate of microfilariae which fail to be taken up by the proper insect host still remains unknown.

DISCUSSION.

These experiments show that microfilariae (*D. immitis*) not only readily pass through the peripheral capillaries but also leave the blood stream and enter the lymphatics. Here again, the question arises as to how far these findings with *Dirofilaria* can be applied to human infection with *Wuchereria bancrofti*. There has been a rather general belief that unsheathed microfilariae are capable of further active migration than sheathed forms, that is, the sheath would act as an impediment. It was shown, however, in a previous paper (DRINKER *et al.* 1935) that sheathed microfilariae (*Loa loa*) are as active and as capable of travel as the unsheathed microfilariae of *Dirofilaria*. KNOTT (1935) encountered only unsheathed microfilariae of *Wuchereria* in a human subject with elephantiasis 2½ days after a blood transfusion with blood from an infected donor. This patient had had no attacks of filaria fever for a year and his blood was negative for microfilaria at the time of the transfusion. This observation would indicate that microfilariae of *Wuchereria* may escape from their sheaths should they become lodged in the capillaries.

In the present experiments, no difference has been noted in the behaviour of microfilariae or in responses of the host to them in either infected or filaria-free hosts. KNOTT, however, has observed that while microfilariae (*Wuchereria*) may pass through the peripheral capillaries of a filaria-free host (man), they failed to do so in a subject showing clinical signs of filariasis.

It was also observed by KNOTT that when microfilariae (*Wuchereria*) are injected into a filaria-free subject they exhibit typical nocturnal periodicity. This finding is significant and runs counter to the theory that the mechanism of filarial periodicity (*W. bancrofti*) is due to a simultaneous daily parturition of the females, involving the destruction daily of as many microfilariae as are produced. It appears that we are still without a satisfactory explanation for filarial periodicity.

SUMMARY.

1. An experiment is reported in which microfilariae (*Dirofilaria immitis*) have been injected into the circulation of an uninfected dog.
2. It has been shown in both donor and recipient that microfilariae readily leave the circulation and enter lymphatics. No evidence of a cellular reaction on the part of the host has been found in relation to living microfilariae.

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RECTAL ANAESTHESIA.

BY

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We have been using rectal anaesthesia for some years in our mission hospitals in Angola. This method has proved so useful and the results so satisfactory that we feel justified in presenting a brief report.

We were led to try out this method by such considerations as the following:—
(1) The cost of the anaesthetic ; (2) The necessity for the surgeon to operate with the help of only partially trained African assistants ; (3) The unfavourable results sometimes met with in the use of chloroform.

The rectal method of administering ether gave promise of meeting the conditions well, since it is economical in the use of ether, simple to administer and relieves the surgeon of the strain of watching the condition of the patient. We have used it in a series of over fifty cases without any unfavourable results. It has proved an ideal anaesthetic in long, tedious operations and in operations on elderly people with weak hearts. It has been used in the following operations : goitre, appendicitis, ovarian tumour, hysterectomy, extra-uterine pregnancy, hernia and amputations. After the operation the patient usually sleeps quietly for some hours. Vomiting is rare and there were no lung complications.

of that encountered in all the tissues of both the donor and recipient of the present study. No cellular reaction on the part of the host has been noted. The entire absence of a foreign body reaction is striking. The fate of microfilariae which fail to be taken up by the proper insect host still remains unknown.

DISCUSSION.

These experiments show that microfilariae (*D. immitis*) not only readily pass through the peripheral capillaries but also leave the blood stream and enter the lymphatics. Here again, the question arises as to how far these findings with *Dirofilaria* can be applied to human infection with *Wuchereria bancrofti*. There has been a rather general belief that unsheathed microfilariae are capable of further active migration than sheathed forms, that is, the sheath would act as an impediment. It was shown, however, in a previous paper (DRINKER *et al.* 1935) that sheathed microfilariae (*Loa loa*) are as active and as capable of travel as the unsheathed microfilariae of *Dirofilaria*. KNOTT (1935) encountered only unsheathed microfilariae of *Wuchereria* in a human subject with elephantiasis 2½ days after a blood transfusion with blood from an infected donor. This patient had had no attacks of filaria fever for a year and his blood was negative for microfilaria at the time of the transfusion. This observation would indicate that microfilariae of *Wuchereria* may escape from their sheaths should they become lodged in the capillaries.

In the present experiments, no difference has been noted in the behaviour of microfilariae or in responses of the host to them in either infected or filaria-free hosts. KNOTT, however, has observed that while microfilariae (*Wuchereria*) may pass through the peripheral capillaries of a filaria-free host (man), they failed to do so in a subject showing clinical signs of filariasis.

It was also observed by KNOTT that when microfilariae (*Wuchereria*) are injected into a filaria-free subject they exhibit typical nocturnal periodicity. This finding is significant and runs counter to the theory that the mechanism of filarial periodicity (*W. bancrofti*) is due to a simultaneous daily parturition of the females, involving the destruction daily of as many microfilariae as are produced. It appears that we are still without a satisfactory explanation for filarial periodicity.

SUMMARY.

1. An experiment is reported in which microfilariae (*Dirofilaria immitis*) have been injected into the circulation of an uninfected dog.

2. It has been shown in both donor and recipient that microfilariae readily leave the circulation and enter lymphatics. No evidence of a cellular reaction on the part of the host has been found in relation to living microfilariae.

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KNOTT, J. (1935). The periodicity of the microfilaria of *Wuchereria bancrofti*. *Ibid.*, xxix, 59.

RECTAL ANAESTHESIA.

BY

H. S. HOLLENBECK, M.D.

Elende, Cuma, Angola, Portuguese West Africa.

We have been using rectal anaesthesia for some years in our mission hospitals in Angola. This method has proved so useful and the results so satisfactory that we feel justified in presenting a brief report.

We were led to try out this method by such considerations as the following: (1) The cost of the anaesthetic; (2) The necessity for the surgeon to operate with the help of only partially trained African assistants; (3) The unfavourable results sometimes met with in the use of chloroform.

The rectal method of administering ether gave promise of meeting the conditions well, since it is economical in the use of ether, simple to administer and relieves the surgeon of the strain of watching the condition of the patient. We have used it in a series of over fifty cases without any unfavourable results. It has proved an ideal anaesthetic in long, tedious operations and in operations on elderly people with weak hearts. It has been used in the following operations: goitre, appendicitis, ovarian tumour, hysterectomy, extra-uterine pregnancy, hernia and amputations. After the operation the patient usually sleeps quietly for some hours. Vomiting is rare and there were no lung complications.

The following is our routine method of procedure in the preparation of the patient and the administration of the anaesthetic :—

1. A cathartic is given the second day before the operation.
2. An enema the night before the operation.
3. A cleansing enema three hours before operation.
4. Morphine sulphate $\frac{1}{4}$ grain one hour before operation.
5. Rectal administration of the following mixture :

Ether 5 to 7 ounces.

Olive oil 2 to 3 ounces.

Paraldehyde 1 to 2 drachms.

Warm the mixture and inject slowly, using a funnel. Begin shortly after the injection of the morphine. Cover the face with a fairly heavy, damp towel. At the conclusion of a short operation the oil and ether mixture may be withdrawn though it may be left in without harm to the patient. It is sometimes necessary to administer a little chloroform or ether by inhalation at the most painful stages of the operation.

This method seems peculiarly adapted to the Africans in our region. They go to sleep quietly and often require no additional anaesthetic by inhalation. In our experience this method does not serve as well for Europeans.

CONCLUSIONS.

1. Rectal anaesthetic is economical of ether.
2. It is easily administered, not requiring special skill.
3. It is safe.

CORRESPONDENCE.

THE HUMAN ORGANISM AND HOT ENVIRONMENTS.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

DEAR SIR,

I read Dr. D. H. K. LEE's article on "The Human Organism and Hot Environments" with very great interest, having myself made some relatively insignificant observations on the same subject, when enjoying for that study, so far as environment goes (to quote Sir CHARLES MARTIN) unrivalled opportunities. I congratulate Dr. LEE on his choice of a subject, and on having secured the support of Sir CHARLES MARTIN in his investigations.

To the four sources of knowledge whence Dr. LEE gleans information, I would like to add another, namely, experiments on animals in tropical climates. It is singular that animals so imperfectly fashioned to resist heat as birds, goats, sheep, cattle, donkeys, horses, etc., should be able to endure exposure to the direct rays of a tropical sun, every day, throughout the summer, with only occasionally a casualty. The secret of this amazing adaptation (so far as my experiments have indicated) seems to lie in ability, acquired by training and "natural selection," to withstand a rise of body temperature that would be lethal to untrained or unacclimatised organisms, and that would be certainly lethal to particular species that seem unable to acquire more than a limited tolerance, even after prolonged training, *e.g.*, hutch rabbits and guineapigs. It is curious that the wild hare is indifferent to extreme heat, while the rabbit even in the wild state, perishes unless it can take refuge in its earth tunnel. I think Dr. LEE's praiseworthy attempt to clarify the subject under discussion by classification of the symptoms and phenomena under four headings, certainly throws these fundamental concepts into relief and enables one to see the trees unobscured by the wood; but in practice (by which I mean "in the treatment of heat-affected men") this simplification might be misleading. Patients suffering from the effects of heat not unusually display the whole gamut of cramps, dehydration, hyperthermia and circulatory embarrassment in parallel or more commonly (to use an electrical metaphor) in series, and the clear-cut clinical entities depicted in Dr. LEE's comprehensive and informing tables are, in my experience, the exception rather than the rule. I am accustomed to regard cramps, dehydration, and heart failure as, in the majority of instances, the prodromal symptoms of hyperthermia, and we are accustomed to ward off the very grave thermal unbalance by grabbing our cases while still in the larval stage of development. SHATTUCK and HILFERTY (1933) found that "heat

exhaustion " as a cause of death from heat effects was the predominating factor in people over 60 years of age. The correlation of heat deaths with increasing age is probably due to progressive diminution in the reserve power of the heart. "Heat exhaustion" in our experience, is common in young people and is correlated, in them, with salt depletion; the associated symptoms of fainting, nausea, low blood pressure, air hunger, cramps, and general collapse being relieved by the administration of salt and water in cool surroundings. For the early recognition of heat-effect cases nothing is more valuable than the examination for chlorides of a 24-hour specimen of urine; and I agree with Dr. LEE's statement (due to TALBOT and MICHELSEN, 1933) that there should be a minimum of 3 grammes of sodium chloride in such a specimen.

I think that the treatment of patients in a random series should be judged on the merits of each case, and I feel Dr. LEE's strictures on the use of glucose and bicarbonate, in addition to saline (which he agrees is theoretically indicated), are rather severe and savour of pedagogy; Squadron-Leader MORTON (1932) conceivably had good reasons (medical, if not physiological) for the inclusion of glucose and bicarbonate in the saline administered to his patients.

The state of electrolyte disequilibrium, or low salt concentration, in the majority of cases, is quite simply due to the steady drain of chloride in the sweat, but under certain easily reproducible conditions, the problem becomes vastly more complex.

CRAMER (1928) found that "Exposure to heat, which inhibits the activity of the adrenal glands, leads to disappearance of the cortical lipid . . ." It is well known that adrenal insufficiency is the prime factor in Addison's disease, and that hypochloraemia and dehydration are important factors in this syndrome (LOEB, 1933). It would seem that in environmental heat we have a predisposing agent for the ionic unbalance that is such a feature of heat effects in man. Advanced pathological opinion is indicated in the statement "It appears, in fact, that one of the major functions of the endocrine system, as a whole, is to maintain the ionic balance of the blood" (HADFIELD and GARROD, 1934). It appears that the parathyroids control calcium, the pituitary water, and the suprarenals sodium. It is significant that the injection of adrenalin produces a marked increase in the lactic acid and glucose content of the blood (CORI, 1931), and this observation provides a possible explanation for the high blood lactate and glucose concentrations found in experimental hyperthermia. It is unfortunate that the careful experiments of RAYMOND WHITEHEAD (1934), who exposed groups of 24 male white mice, 91 to 112 days' old at death, in a room at 34° to 38° C., for 3 days (22 per cent. died after 1 day, 8 per cent. after 2 days, 1 per cent. after 3 days), showed that there was no significant divergence from normal in the relation of lipid-laden cortex to area of permanent cortex.

There is no doubt, to my mind, of the reality and importance of acclimatisation for men and animals that live and work in hot environments. In the case of man (assuming that the organism under training is physically fit, has the

proper number of sweat glands per unit of skin surface, and is in the prime of life) the factors are, briefly :—

1. *Proper hygienic education* which may be acquired in the “Hard school of experience,” or better, by precept, based on well known principles of prophylaxis, to wit, suitable clothing and headgear, ample salt and water supply, light meals taken in the cool part of the day, extreme moderation in alcohol consumption, sensible precautions to avoid intercurrent disease, short working hours and cool living quarters.

2. *Psychological education, i.e.,* trained disregard of unavoidable discomfort, loss of sleep and unpleasant visceral sensations, in the way that the soldier is trained to disregard fatigue, discomfort, fear, etc., and as the trained athlete undertakes feats of endurance that involve gruelling work. In my experience fear of heat effects is, on occasion, a very important factor in their causation and exacerbation. In this connection the work of Miss GLOCK (1935) is of great interest. This worker found that a subject who liked heat was unaffected by exposure to an artificial hot climate, and seemed acclimatised from the start; while another subject, who heartily disliked heat, showed marked physiological reactions to the artificial climate. Miss GLOCK suggested that all subjects who contemplated a career in hot climates, should be put through a course in an artificial climate and their reactions noted for future reference with the object of ultimately selecting suitable candidates for the tropics or other hot environments.

3. *Gradual introduction to the most severe effects of the climate* in the manner chosen by the Union of South Africa (1933) observers in the Witwatersrand Gold Mines who consider that 14 days is sufficient to train the most backward candidate for acclimatisation under the peculiar conditions that exist there. The experiments of MILLS and OGLE (1933) on animals pointed, broadly speaking, to the same conclusions. In the climate limned by the temperature chart (MARSH, 1933), shade temperatures begin to increase at the end of March; from the beginning of April, until the first week in June, there is a wavering ascent of temperature, not unlike the “staircase” chart of a patient during the first week of typhoid fever. From the middle of June until the end of August, the “fastigium” (still on the analogy of typhoid) is maintained, the daily excursions of the maximum shade temperature being of comparatively small magnitude; about the middle of September “defervescence” begins, and is complete by the middle of November, the characteristic intermissions being again in evidence. Our period of training for acclimatisation is prolonged, being about 8 weeks—four times the length of time considered the maximum by the South African authorities; our period of training, however, is that laid down by Nature, who, in her wisdom, repeats the course every year. For those not in the prime of life, or suffering from intercurrent disorder, the effects of faulty hygiene, insufficient training, or some hitherto undiscovered fundamental defect, shelter from the most severe effects of the climate is necessary,

to enable them to recover sufficiently to renew the fight with the elements or to succour and refuge them while arrangements can be made for their evacuation. Such a refuge is best provided by some such arrangement as the artificially cooled ward described before (RENNIE, 1930 ; MARSH, 1930).

Modern cold storage plant provides us with a temporary refuge from hot environments ; air conditioned living quarters are the next step in the struggle for the amenities of life.

I agree that much remains to be done to explain the reactions in the human organism in hot climates, but most of the causative factors are known, and illness due to heat can be prevented, and when occurring can be efficiently treated, with the knowledge at our disposal now if it is correctly used by the proper authorities.

I am, etc.,

FRANK MARSH.

c/o A.I.O.C.,
Masjid-i-Sulieman,
via Ahwaz, South Persia.
29th July, 1935.

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BANCROFTIAN FILARIASIS.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

SIR,

CLAYTON LANE, in a note on periodic Bancroftian filariasis, appearing in the last number of these TRANSACTIONS (1935, xxix, p. 135), in attempting to measure the value of papers on infection with periodical *Wuchereria bancrofti*, quoted my paper in an earlier number (1935, xxviii, 613) but I am afraid he missed the main point of my views, and in reporting parts of my conclusions he neglects others in such a manner as to alter entirely my meaning.

(1) I never denied that adult worms can be found elsewhere than in the sites I quoted. I simply said that I have been unable to find adult worms in any situation other than the lymphatics of the cord, unless the worms have been the cause of important clinical manifestations.

(2) The theory I advanced is that the *normal* habitat of the worm, that is where the worm develops and reproduces in *normal* circumstances, is a definite part of the genital organs. I have been very particular in writing "*normal*" in italics, meaning by normal the cycle of the worm in individuals harbouring *Filaria bancrofti* without showing any clinical symptoms and before the appearances of complications or later developments due to the filarial infection.

(3) It is not even questioned that adult worms, living, dead or calcified, can be found in other localisations than those I quoted, and in the "focal spots." That that is so is already made clear in my paper (page 625), where I consider as the most important factor in causing the later developments of the disease "*the emigration of the adult worm from its normal habitat, or its development elsewhere than in the genital area.*"

CLAYTON LANE will admit, I hope, the fact that "focal spots" are one of the manifestations in filariasis which are more advanced than those I considered under my "*normal*."

(4) Another point to which I must draw his attention relates to the lymph glands. In my paper I spoke of the lymph glands draining the genital organs. CLAYTON LANE adds on his own account, between parentheses, "which are the lumbar glands." I was quite well aware that "the draining of lymph from the epididimus and cord (in normal anatomy) is not into the inguinal glands, nor have I made any such statement. I purposely mentioned "genital organs," meaning by this definition the whole of the genitalia, which are composed of different parts, whose lymphatic system is somewhat more complicated than the simple draining of lymph from the epididimus and cord to the lumbar glands.

As I stated in the introduction to my paper, it was only a resumé of the

main conclusions of my researches, so I was not able to bring forward all my evidence and all the details, which will be given in due time.

I am, etc.,

Georgetown,
British Guiana.

CESARE ROMITI.

2nd September, 1935.

BANCROFTIAN FILARIASIS: A WRONG INFERENCE AND RIGHT CONCLUSIONS.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

SIR,

In the number of these TRANSACTIONS issued in July of this year, I questioned whether Dr. JAMES KNOTT, of the Virgin Islands of the United States, had used the same diagnostic technique before and after injection of microfilariae into a man who was believed to be free from the infection at the time when this transfusion was made. KNOTT writes to me, under date 9th September, 1935, that he did in fact use the same technique before and after the injection of microfilariae. Any inferences which may be drawn on the contrary supposition are then incorrect and among them my suggestion (p. 140) that his case E.D. might be explained in this way. KNOTT adds the fact that one man into whom he transfused microfilariae on 28th March, 1934, was still showing them in the blood at the time he wrote. I believe it is not prejudice which makes me urge that such injections in an endemic area are not safe foundations for conclusions, particularly in face of MURGATROYD's experience to the contrary in this country, inconclusive as he suggested it at the time to be. Clearly it is an experiment which needs repetition in a non-endemic place. In making the proposal that a particular type of experiment is unconvincing when carried out in an endemic country, there is no suggestion of lack of appreciation of the great value of the work which KNOTT has put, and is putting, through with keenness and skill. One is justified in looking with pleasurable anticipation to the results of the active collaboration which will have begun before this is in print between himself and Prof. F. W. O'CONNOR of Columbia University, though regretting that circumstances appear to have prevented the aid which had been hoped for from Dr. DONALD L. AUGUSTINE of Harvard University.

As to ROMITI's letter in this number of the TRANSACTIONS, any idea of misrepresenting him or of using against his case any of the words in which it was expressed was not in my mind; but even now his meaning is no clearer to me. Yet he does say plainly that, apart from the varicolymphocoele of the cord

and cystic formations in the plexus, he has not been able to find adult living worms anywhere (p. 622, item 5). My reply was that his observations are imperfect and that such worms have been shown at this Society's meetings. In the first part of his paper ROMITI said that it was only intended as a resumé of his findings, but did not make it clear that they were to be published. The matter is better left till the facts are fully stated. As they are now known I find no need to alter any of my views.

It remains to add that work which, it is understood, is planned in West Africa, and has been begun in Madras, as T. BHASKARA MENON's *Maharaja of Travancore Curzon Lectures* tell, shows that the British Empire is also interested in the active explanation of Bancroftian filariasis, and to point out that the British Isles have no endemic filariasis, but are conveniently visited by those who suffer from this infection.

I am, etc.,

CLAYTON LANE.

AVITAMINOSIS B₂.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

DEAR SIR,

I have been much interested in the paper on "Avitaminosis B₂" by J. V. LANDOR and R. A. PALLISTER in these TRANSACTIONS, Vol. xxix, No. 2, pp. 121-133.

The description of the clinical aspects of this disease, as it occurs in the prisons in Malaya, as well as the account given by L. NICHOLLS, of Ceylon prisoners in 1933, tallies exactly with a sore tongue and mouth disease which I observed in the prisons of Ceylon in 1912 and which I described and figured in my monograph, "A Report upon Researches in Sprue in Ceylon," Cambridge University Press, 1915, pp. 46-48. The excoriated appearance of the tongue, lips and angles of the mouth are shown in Plate I, Figs. 15 and 17 of that monograph.

I thought at the time that this might be a manifestation of the sprue process localized to the mouth, and I found it in 181 out of 1,461 natives of all races that I examined—Sinhalese, Tamils, Javanese and Malays. It appeared to have no relation to diarrhoea or intestinal disturbance and to be especially prevalent in prisoners in the jails of the Southern Province. It is interesting to note that the frequency of sore tongue disease was ascribed at the time by prison medical officers to a diet of salt fish and there was also evidence to show that when once it made its appearance in a jail other cases would soon declare themselves.

The possibility of the relationship of this glossitis being one aspect of the pellagra symptom-complex was considered at the time. It is interesting to realize that this disease is now being recognized as being in some way related to pellagra and to be associated eventually with lesions of the central nervous system.

149, *Harley Street*,
London,
12th September, 1935.

I am, etc.,

PHILIP MANSON-BAHR.

ATEBRIN MUSONATE.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

DEAR SIR,

My attention has just been drawn to an article in these TRANSACTIONS (1935, xxix, 103) describing a demonstration given before the Society by Dr. S. SOMASUNDRAM.

Without wishing to criticize the scientific conclusions drawn from the Kandy experiment there is one point which cannot go unchallenged and that is on what authority the author states that I have ever claimed that one injection of atebtrin musonate could effect a cure of malària. My first and only paper in this connection, " Preliminary observations on a new soluble atebtrin compound " (*Indian Medical Gazette*, 1935, lxx, 185) proves that two injections are necessary. That I treated a few cases experimentally with one injection as discussed in my paper can hardly be called a claim.

May I make it clear that I have never claimed anything in this connection.

I am, etc.,

A. T. W. SIMEONS.

Khatau Mansion,
Cooperage, Bombay.
10th September, 1935.

IN the death of their
MOST GRACIOUS PATRON,
HIS MAJESTY
KING GEORGE V,

Fellows of the Royal Society
of Tropical Medicine and
Hygiene throughout the
world have suffered an
irreparable loss, and take this
opportunity of expressing
their profound grief at the
passing of one who has
always followed with the
greatest interest the progress
of the Society and the
developments in Tropical
Medicine upon which the
happiness and prosperity of
tropical lands so much
depend.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

VOL. XXIX. No. 4.

Proceedings of an **Ordinary Meeting of the Society** held at
Manson House, 26, Portland Place, London, W.1, at 8.15 p.m., on
Thursday, 21st November, 1935.

Sir ARTHUR BAGSHAWE, C.M.G., M.B., D.P.H., *President*, in the Chair.

The President : In calling on Dr. DE VERTEUIL to read his paper I should like to say that we are fortunate in having here this evening a distinguished Fellow of the Society from Brazil, Dr. MAGARINOS TORRES of the Instituto Oswaldo Cruz. I hope he will take part later in the discussion.

PAPER.

THE STUDY AND CONTROL OF PARALYTIC RABIES
TRANSMITTED BY BATS IN TRINIDAD, BRITISH WEST INDIES.

BY
ERIC DE VERTEUIL, M.D., B.S. (LOND.), M.R.C.S., L.R.C.P., D.P.H. (LOND.),
AND
F. W. URICH, F.R.E.S. (LOND.).

INTRODUCTION.

This paper is the result of team work carried out by a number of Trinidad-born men.

J. L. PAWAN, M.B.E., M.B., Ch.B., D.P.H., Government Bacteriologist, has been responsible for the bacteriological and experimental section of the work and to him is due the credit of having discovered Negri bodies in the brains of animals, human beings and bats in Trinidad in 1931. He has received material assistance at various times from the Lister Institute in London and from the Rockefeller Institute of New York, and particularly from E. WESTON HURST, M.D., D.Sc., of the Lister Institute, with whom he was associated in publishing in the *Lancet* two papers on the outbreak of rabies in Trinidad (HURST and PAWAN, 1931 and 1932). Prof. F. W. URICH, F.R.E.S., has been responsible for the zoological side since January, 1934, and his unique knowledge

of the fauna of Trinidad proved to be an enormous asset in studying the habits of the bats in the field, both when at home and on their feeding grounds, as well as in rearing the bats in confinement for the purpose of observation and of experimental work. Capt. H. V. METIVIER, B.Sc., M.R.C.V.S., Government Veterinary Surgeon, has been responsible for the veterinary section, and has worked in close association with Dr. PAWAN in connection with the experimental investigation on animals and bats. Much valuable work has also been carried out in this section at the Weybridge Veterinary Laboratory under W. HORNER ANDREWS, D.Sc., M.R.C.V.S., particularly in connection with the production of anti-rabic vaccine.

The Public Health Section of the work has fallen to the lot of C. F. LASSALLE, M.D., D.P.H., former Deputy Surgeon-General and Medical Inspector of Health until 1933 and subsequently to one of us (E. DE V.), whilst acting in the same capacity from May, 1933, to May, 1935. During the past year, as a result of the joint activities of Professor URICH and of the Health Department, in searching out the feeding, digesting and sleeping places and investigating the habits of bats in general and of *Desmodus rufus* in particular, a large amount of valuable information in connection with the epidemiology and control of the disease has recently come to hand, and it is in connection with this section of the work that Dr. DE VERTEUIL has been mainly responsible, whilst working in close association with his other colleagues, especially Professor URICH. We are particularly grateful to the Surgeon-General, Dr. K. S. WISE, and to E. J. WORTLEY, C.M.G., Director of Agriculture, for their continued interest and support in connection with this investigation and for permission to publish.

We are also especially indebted to Sanitary Inspector L. B. ASSANG, who was responsible for most of the spade work, especially at night under trying and difficult conditions, as well as to Mr. L. WEHEKIND and Mr. E. J. ACHE, who all assisted materially in developing the methods of investigation and capture of the bats in the field.

We propose to deal with the subject matter under the following heads :—

- I. History of the vampire bat.
- II. History of paralytic rabies in Brazil and Trinidad.
- III. Short summary of the incidence, mortality and symptoms of human paralytic rabies in Trinidad 1929-1935.
- IV. Study of the bat fauna of Trinidad, and of *Desmodus rufus* in particular.
- V. Bat destruction with special reference to methods of destruction and capture of *Desmodus rufus*.
- VI. Laboratory experiments and results of examination of bats' brains for Negri bodies.
- VII. Epidemiology and control of paralytic rabies.
- VIII. Conclusion.

I. HISTORY OF THE VAMPIRE BAT.

At the present time the blood-sucking, or more correctly speaking, blood-lapping vampire bats, *Desmodus*, are under investigation in three different portions of the New World as being the carriers of two distinct diseases.

These bats have long been known to be a source of annoyance and of fear in several of the South American countries ; and long before the days of DARWIN and the voyage of the "Beagle," numerous imaginative stories had been written of the wonderful power of these bats to fan their victims to sleep as a preliminary to a painless bite and the subsequent blood-sucking process. CHARLES DARWIN was the first scientist to observe a vampire in the act of drawing blood and to note its procedure with fair accuracy, but it was left to L. H. DUNN of Panama to record in 1932 that the vampire is a blood lapper and not a blood sucker. It is now also further known from the observations of several scientists in the field as well as from recent work in Trinidad and in Panama, that the vampire does not hover over, but actually walks slowly and stealthily to, his victim before biting him. A very recent publication in *Zoologica* by DITMARS and GREENHALL (1935) gives a complete review of the history of the bat and a description of its habits. The photographs are particularly interesting.

At no time, however, until recently was it suspected that this elusive and interesting mammal might be the carrier of disease to man and beast ; and this, no doubt, accounts to a large extent for the comparatively scant literature which is available in connection with the life history of *Desmodus* and indeed of bats in general.

In Panama, equine trypanosomiasis (also called "murrina" or "derengadera") has been recognised since 1910 and various insect carriers (horse flies, ticks, mosquitoes, and the reduviid bug *Triatoma geniculata*) have been suspected as being the natural vectors of the disease (CLARK, CASSERLY and GLADISH, 1933). During the past two years, however, the vampire bat, *Desmodus rotundus murinus* Wagner, has been incriminated as being the main vector that keeps the disease enzootic, and it is believed that it is able to disseminate the disease on its nightly feedings over an average period of about one month after it has acquired the disease and before death overtakes it as a result of the disease.

The question of controlling the incidence of equine trypanosomiasis by controlling the *Desmodus* population now appears to be engaging the serious attention of the Panama Health Authorities. In Trinidad and in Brazil, on the other hand, *Desmodus rufus* and *Desmodus rotundus* have been definitely incriminated as the principal vectors of paralytic rabies ; but whilst in Brazil the disease appears to be confined solely to stock animals, it has been the cause of a great deal of anxiety to the Health Authorities in Trinidad on account of its incidence amongst human beings as well as among livestock.

So far as the existence of vampire bats in Trinidad is concerned, Professor CAMERON of McGill University has informed me that he has read somewhere

that bat bites were reported as occurring in Trinidad during the visit of discovery of the island by Columbus in 1498—at any rate there is ample evidence that they have been in the island since the early days of the 19th century.

II. HISTORY OF PARALYTIC RABIES IN BRAZIL AND TRINIDAD.

Paralytic Rabies in Brazil.

The story of paralytic rabies is an interesting and fascinating one, and dates back to the year 1906 when the disease first broke out in epizootic form among cattle and other livestock in the State of Santa Catarina in Brazil, along a narrow stretch of land between the sea and the mountains.

Since 1906, it has persisted there in sporadic form with occasional severe epizootic outbreaks. The true nature of the disease and its method of spread does not appear however to have been recognized until about 1921, when HAUPT and REHAAG (1921) published a paper describing the outbreak and proving, after experimental investigation of a bat caught biting a cow in the day-time, that the disease was rabies, and that the vector was the vampire bat. No rabid dogs were known to exist, and wholesale destruction of dogs made no impression on the case incidence.

It was then noticed that a swiftly flowing river, impassable to dogs, had proved to be no barrier to the spread of infection and that the heaviest mortality occurred in the most thickly wooded districts where vampire bats were most prevalent, and where cattle bitten by such vampires during the day-time afterwards developed rabies. Subsequently, between 1931 and 1934, severe epizootics claiming sometimes 60 per cent. of the cattle as victims, broke out in the State of Matto Grosso as well as at Santa Catarina; and the vampire bat *Desmodus rotundus* was conclusively proved to be the vector. (Institute of Animal Biology, Brazil, 1934.)

Preventive treatment was confined to the use of anti-rabic vaccine made from *virus fixe*, which is stated to have given perfect immunity: 112,000 animals were immunized during 1931 and 1932.

No systematic attempt is reported to have been made to control the disease by controlling the *Desmodus* population, though bats in caves and hollow trees were sometimes destroyed.

The affected animals showed the usual symptoms of paralytic rabies; which, it should be noticed, is the normal type in all herbivorous animals and rodents bitten by rabid dogs.

The symptoms were briefly grinding of the teeth, tremulous and involuntary movements, muscular incoordination, salivation, paralysis and respiratory failure.

Paralytic Rabies in Trinidad.

Paralytic rabies first occurred in livestock in Trinidad in 1925 when 60 animals died in and around Port-of-Spain, the capital of the island, within

a few months. In subsequent years epidemic outbreaks have occurred in most parts of the island and altogether a few thousand animals are estimated to have died of the disease between 1925 and the present date, the disease occurring principally during the beginning of the rainy season and having a mortality rate of 100 per cent. No case of rabies in dogs or other carnivorous animals has been recognized since 1914 and the quarantine laws have been rigidly enforced.

The disease was at first diagnosed as botulism on clinical and bacteriological grounds; but subsequently, after an outbreak in 1929, among human beings of thirteen cases of acute myelitis (then diagnosed as acute anterior poliomyelitis), four more cases in 1930 and one in 1931, it was established by PAWAN and HURST that the human and animal diseases were similar and were due to the virus of rabies with the vampire bat as the probable vector (HURST and PAWAN, 1931). Shortly after this, taking the clue from the Brazilian workers, PAWAN was also able to prove, by histological and experimental examinations, the infection with rabies of twelve bats which had been caught during the day-time either attempting to bite animals or biting each other whilst flying around. These twelve bats were distributed between two species—*Artibeus planirostris trinitatis* (fruit-eating) and *Desmodus rufus* (blood-lapping).

Five more human cases occurred in 1932, and in 1933 two cases were diagnosed clinically, but the bacteriological examination failed to reveal the presence of the virus of rabies.

The symptoms in all cases showed clearly that the human disease was a definite clinical entity—all the cases being of the paralytic type of rabies—thus constituting an occurrence unprecedented in any other part of the world in respect of the incidence of this type of rabies.

The control methods then in use were :—

1. In the case of *animals*—anti-rabic vaccine and protection from bat-bites by bat-proofing stables and keeping lighted lanterns therein at night. In 1932, 6,000 animals were immunized, 5,709 in 1933, 12,703 in 1934, and 3,174 from January to June, 1935, by the Government Veterinary Department.

2. In the case of *human beings*—control methods were confined to

- A. Protection from bat-bites by

- (a) the use of lights at night and hanging spiky branches in upper parts of rooms and galleries;

- (b) protection of sleeping rooms by wire screening all openings;

- (c) the use of mosquito nets over beds;

- (d) the destruction of bats generally and especially those of unusual habits.

- B. Protective inoculation of bat-bitten persons with anti-rabic vaccine made from *virus fixe*.

These control methods did not prove satisfactory and as a result of the recommendations of a Committee appointed by the Governor in October, 1932, to enquire into the incidence of the disease and to make suggestions for its control, Professor F. W. URICH was eventually appointed in January, 1934, to study the relation of bats to the disease and to develop methods for their control. Between January and August, 1934, while the animal disease existed

over an area of about 150 square miles, seven more human cases occurred, all in a small confined area of about 9 square miles, and it was then felt by the Health Department that valuable information might be obtained by carrying out on the following lines an intensive epidemiological survey in selected infected areas in connection with (A) Human beings, (B) Bats, and (C) Animals and Poultry.

A. Human beings.

(1) House to house inspection in connection with

- (a) villages, estates and estate barracks and groups of isolated houses ;
- (b) recent bat-bites among human beings—with special reference to their association with bat-bites among animals and poultry and the proximity of roosting and digesting places of *Desmodus* bats.

(2) Enquiries in connection with any possible missed cases of rabies in out-of-the-way districts.

B. Bats.

(1) Searching for the night-time digesting places of *Desmodus* bats under bridges, culverts and other places. These are easily recognised by finding the typical tarry excrement of the bats.

(2) Searching for the day-time roosting and sleeping places of *Desmodus* in caves, hollow trees, underground passages, etc.

(3) Collection of bats other than *Desmodus* where considered unusual or uncommon.

(4) Trapping of live *Desmodus* bats while feeding on animals or in their sleeping or digesting places and observing their habits and general behaviour in the field.

(5) Caging, artificially feeding and observing the habits and behaviour of *Desmodus* in captivity.

(6) Special observation of any abnormal or presumably rabid *Desmodus* or other bat outside or in captivity so as to develop a knowledge of the symptoms presented (if any) by such rabid bats.

C. Animals and Poultry.

(1) Number of animals and poultry bitten by bats.

(2) Proximity of digesting and roosting places of *Desmodus* to collections of stock and poultry in various portions of the different districts.

(3) Number of stock animals dying of rabies in relation to the number of bat-bites amongst animals and man in the immediate neighbourhood, as well as to the number of *Desmodus* roosting and digesting places.

In addition to this intensive survey in these selected rabies-infected areas, valuable information was also obtained at the same time from sanitary inspectors in non-infected areas in connection with the normal number of bat-bites of animals, poultry and men in the case of presumably non-infected bats.

The results of this survey proved to be so encouraging that by July, 1935, nine trained rabies inspectors were actively pursuing bat destruction work (especially aimed at *Desmodus rufus*) in the various infected rural districts under the Public Health Department. Meanwhile the City of Port-of-Spain had also organized a trained squad of inspectors for the destruction of bats in and around the immediate outskirts of the city. All this work is being carried on with the close co-operation of Professor URICH and of the Bacteriological and Veterinary Departments.

III. SHORT SUMMARY OF THE INCIDENCE, MORTALITY AND SYMPTOMS OF HUMAN PARALYTIC RABIES IN TRINIDAD FROM 1929 TO 1935.

INCIDENCE.

The following Table shows the incidence of cases by the month, as well as the number of cases and the duration of the outbreak, wherever this has occurred in epidemic form in a district.

TABLE I.

Monthly Incidence of Cases.														Local Epidemic Outbreak.	
Year.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total.	No. of Cases.	Duration of Epidemic.
1929	0	0	0	0	0	0	7	4	2	0	0	0	13	13	10 weeks
1930	0	0	0	0	0	0	1	3	0	0	0	0	4	3	3 weeks
1931	1	0	0	0	0	0	0	2	1	0	0	0	3	0	No epidemic
1932	1	0	3	0	0	0	0	0	1	0	0	0	5	3	1 week
1933*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	No epidemic
1934	1	0	2	0	0	0	0	4	0	0	0	0	7	4	3 weeks
1935															
(Jan.-July)	0	0	2	6	10	4	0	0	0	0	0	0	22	13 7	6 weeks 5 weeks
Total	3	0	7	6	10	4	8	13	4	0	0	0	55	43	1 to 10 weeks

* Two clinical cases occurred, but the diagnosis was not confirmed by bacteriological investigation.

From the above Table it will be noticed :—

1. That the disease usually occurs in small local epidemic outbreaks over a comparatively short period of from 1 to 10 weeks—isolated endemic cases continue to occur between the epidemics.

2. That 64 per cent. of the cases occurred between May and August, which corresponds to the first 4 months of the rainy season.

3. That no cases occurred in February, October, November and December.

4. That the greatest number of cases occurring in any one epidemic has been 13.

Age Incidence.

Age in years.	0-5	5-10	10-15	15-20	20-30	over 30	Total.
No. of cases	2	14	12	6	8	13	55

N.B.—47 per cent. occurred in children between ages 5 to 15.

Sex Incidence.

Thirty-seven cases occurred among males (= 67 per cent.) ; and 18 among females ; total 55 cases.

Race Incidence.

The cases occurred among blacks, East Indians and other coloured persons. No cases occurred among white people.

As bat-bites occur indiscriminately among men, women and children of any colour or race whenever sufficient facilities are presented, too much significance should not be attached to the above figures ; it would appear, however, that children from 0-5 are more often protected by night-lights and mosquito nets than are the older children.

Mortality Rate.

The mortality rate is 100 per cent.

Symptoms.

After an incubation period of about 3 to 4 weeks, the symptoms usually come on after one bat-bite only and vary according to the point of entry of the virus into the body and are usually first recognized by an initial fever of 2 or 3 days' duration with marked sensory disturbances, such as tingling, numbness or burning gradually spreading upwards on the parts affected. In the case of bat-bites of the upper or lower extremities, paresis of the affected limb with eventual flaccid paralysis of both sides follows within the next few days : with lower extremity lesions, which are by far the commonest, retention of urine (sometimes the first reported symptom), abdominal distension, constipation, salivation and paralysis of the upper limbs appear gradually as the lesion spreads up the cord to terminate the illness in failure of respiration usually on about the 7th day. Although in a small series of cases HURST and PAWAN encountered no instance of typical clinical rabies, such cases do occur with bat-bites on the face and forehead when muttering delirium and restlessness are prominent features. In these cases as well as in most of the upper extremity cases, incontinence of urine and faeces does not occur, and knee reflexes may remain unimpaired to the end. Hydrophobia has not been recognized as a usual symptom, but in some of the cases it has been a marked feature. The longer duration of the disease, compared with 3 or 4 days for the ordinary form of hydrophobia, as well as the usual absence of early maniacal excitement and general muscular spasm should be specially noted.

IV. STUDY OF THE BAT FAUNA OF TRINIDAD AND OF *Desmodus rufus* IN PARTICULAR.

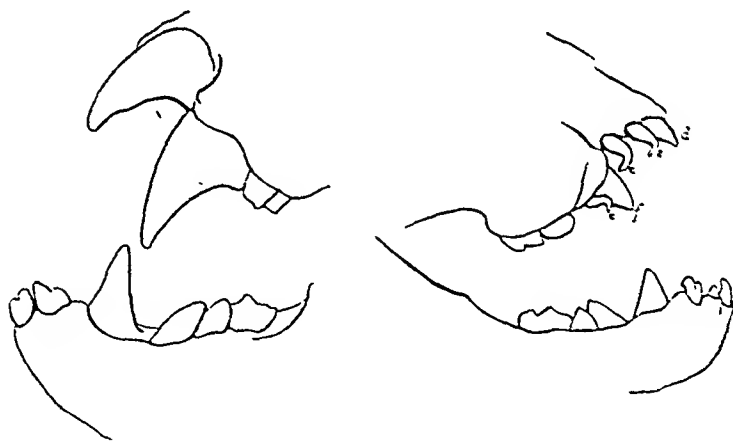
The bat fauna of Trinidad consists of 34 species distributed among 9 families and 29 genera. The majority are insect and fruit eaters, but in addition there are fish eating bats, flower visitors, flesh eaters, and one blood lapper—*Desmodus rufus* (Wied).

CHARACTERISTICS AND LIFE HISTORY OF *Desmodus rufus*.*Characteristics.*

Desmodus has the peculiarity of being the only mammal that is highly specialized to take only blood as food. It is fairly well distributed throughout the Island of Trinidad, but appears to be especially prevalent near the sea, near the banks of the rivers, and in mountain caves. It measures about 13 inches (330 mm.) from wing tip to wing tip. The male has a breadth of wing of 80 mm., whilst the head and body measure about 71 mm. on the ventral surface. The wings of the female expand to about 105 mm. and the head and body measures about 73 mm. *Desmodus* belongs to the group of bats that have no nose leaf and no tail, and have the interfemoral membrane so much reduced as to be almost non-existent. The best distinguishing feature, however, is the dental formula $I \frac{1}{2} C \frac{1}{1} P \frac{1}{2} M \frac{1}{1}$, making a total number of 20 teeth. These teeth cannot crush and are especially adapted for cutting.

DENTITION OF *DESMODUS RUFUS* BLOOD-LAPPING BAT.FIG. 1.—Adult *Desmodus rufus*.

- (a) Triangular cutting portion of upper incisors—used for gouging out the required wound for lapping blood.
- (b) Upper canine teeth—used as props or supports to allow the gouging movement of upper jaw.
- (c) Groove in lower jaw through which the tongue moves and the blood flows during blood lapping. The lower jaw does not enter into the biting or rather gouging out process.

FIG. 2.—Baby *Desmodus rufus*.

- (d) Growing permanent incisor.
- (e) Milk teeth used for holding on to mother.
- (f) Growing permanent canine.

Drawings by O. Atteck.

In colour the males and females vary slightly. On the back both sexes are grey or reddish brown, whilst on the ventral surface the colour of both sexes is very light brown, almost white, but the males have a silvery gray sheen which the females have not.

Desmodus has a narrow oesophagus and hardly any stomach, the alimentary tract from mouth to rectum being nothing else but a fairly narrow tube with, of course, the usual appendages and convolutions.

Life History, Reproduction and Growth.

Desmodus produces only one young at a time. The period of gestation has not yet been positively ascertained, but is not less than 3 months. Bats with advanced embryos have been caught in January, June and September, and young bats were also born in captivity in November, whilst half-grown bats were observed in January and September. From the above data it seems obvious that breeding goes on all the year round.

When born the young bat is almost naked, but is quickly covered with soft brown hair. At first it usually clings to the front part of the mother's body, but hangs independently as it gets older; and towards the end of its adolescence it is left at home, as indeed is the habit of most bats. The milk teeth are in the shape of minute spicules whose chief function seems to be for holding on to the body and teats of the mother. It lives at first on the mother's milk only, but as blood enters into its diet it appears likely that it accompanies the mother on her nightly rounds. (Figs. 2 and 6.)

Bat-bites among animals.

The food of *Desmodus* seems to be confined to the blood of warm-blooded animals, including man, most domesticated mammals and poultry; cats, however, appear to escape. Cattle of all ages, the horse kind, goats, sheep, pigs and dogs are their usual victims. Among pigs the teats of sows are frequently bitten. On horses, cattle, sheep and goats the favourite places for biting are the shoulder and back of the neck and to a less degree the flanks and legs, whilst in the case of poultry the combs of cocks, and the legs and back of the neck under the feathers of fowls are the places mostly selected. Dark coloured animals are usually preferred and in the case of black and white animals the black patches are picked out for biting.

After recording the bites daily among about 200 animals at the Government Farm for a period of about 4 months, the Government Veterinary Surgeon reported the following results:—

1. The zebu cattle (white coats) are as a rule never bitten, both when kept in a separate savannah and when mixed with animals with coloured coats.
2. In the pure bred holsteins and grade holsteins the blacker the coat, the more frequent the bites.
3. Coloured (black, brown, etc.) and white animals are generally bitten on the black and brown spots.

Bat-bites among Human beings.

In several non-infected areas human bat-bites were found to be much more frequent than was thought likely, and in some instances these appeared to take place on a larger scale than usual in recently infected areas (*i.e.* in areas where animal rabies was recently reported), two or three persons being bitten on the same night in the same house. In one such village out of 30 houses visited 20 persons (adults and children) showed evidence of having been bitten within the past 2 or 3 weeks; while a visit to the village school elicited the information that 10 out of a group of 30 children had also been bitten within the past 3 weeks.

In another instance a child aged 4 had been bitten on seven different places in one night, whilst an adult was bitten eleven times, principally on the face. During this period the livestock which was still available in the close vicinity was also being freely bitten. (Fig. 9.)

This increase in the frequency of biting of animals and persons has been used as an index in attempting to locate the direction of spread of the disease, taking the view that such bats are infected bats which have changed their habits. In this connection it should be mentioned that a fair number of *Desmodus* bats have been caught biting animals in the day-time, and that the large majority of these so-called "daylight" bats have proved to be infected.

On the other hand, most of the persons so bitten are known not to have contracted paralytic rabies; and the explanation may lie rather in the fact that through fear of the disease in a recently infected area bat-bites are more likely to be reported, and that the bat-proofing of animal sheds, which usually corresponds with the introduction of the disease in an area, is obviously liable to lead to an increase in human bat-bites—whilst many of the animals are also commonly removed to non-infected areas by their owners.

At the small island resorts of Monos and Gasparee (where no stock animals are usually kept) biting of poultry and of human beings by bats has always been known to be of nightly occurrence and it has long been recognised that where the poultry is protected from bats the human beings are much more likely to be bitten.

From the above indications, therefore, it would appear that biting of human beings in both infected and non-infected areas will usually take place only when and where the usual blood supply from animals and poultry is suddenly cut off, such as does occur after the death of numerous livestock in an infected area; where obvious facilities, however, for biting persons are offered to the bats at any time, such tit-bits would presumably not be refused. The fact that animal cases of rabies in large numbers have invariably preceded the human cases points to the same conclusion. This latter fact is a valuable and timely warning signal for the control of human paralytic rabies in recently infected districts. A census of bat-bites taken in the City of Port-of-Spain (population about 72,000) showed that only two persons were bitten between 1st March and 31st July, 1935, in spite of the fact that a fair number of bats were caught both in the city and in its immediate vicinity.

Mode of Attack.

Most bats when on the ground shuffle along with outstretched wings, which they use for locomotion ; but *Desmodus* is an expert walker and progresses on all fours. The thumb is long and is provided with pads whilst the feet are flat. Its wings fold very compactly close to the body in umbrella-like fashion, so that the fore limbs are free and are in this way wonderfully well adapted for walking.

The following observations on the mode of attack on a cock and on a goat were made recently in our bat house. In the case of the cock, a *Desmodus* was observed about 9 a.m. feeding on its heel. The cock did not appear to be inconvenienced in any way and stood still for about 12 minutes whilst the bat lapped the blood as it slowly trickled down. After this time, however, the cock became restless and walked away followed closely by the bat, which eventually retired into a corner after a second unsuccessful attempt at further lapping.

In the case of the goat the bat had been kept fasting for 48 hours, when a white goat was introduced at about 5 p.m. The bat, which was then hanging on the roof of the bat house, flew down to the floor and walked leisurely towards the goat. After a short interval it pitched on to the back of the goat and walked astride towards the shoulder where, after biting off some hair to clear a small space, it made a quick downward gouging motion by means of its upper incisors and soon after the blood was seen to ooze out. The goat was apparently hardly conscious of the bite. The bat then lapped the blood for about 10 minutes and flew down to the floor, where it remained for a few more minutes before flying up to the roof. During the lapping the tongue could be seen to move forwards and backwards like a piston, whilst the blood poured through a small groove in the lower lip. The wound made is of a shallow crater-like nature and about $\frac{1}{4}$ inch long and $\frac{1}{8}$ inch wide. (Fig. 7.)

Digesting Places.

Desmodus is a child of darkness and leaves its sleeping place only after dark. The first meal of blood is taken early in the night and the bat engorges to such an extent that flight becomes laboured and it resorts to a near-by digesting place, often a stable, an abandoned uninhabited room or more usually the under portion of a bridge. The typical tarry excrement left in these places provides a valuable indication in connection with control work. (Fig. 8.)

Sleeping Places.

The large majority of *Desmodus* bats have been captured during the day in caves and hollow trees (as many as 117 from one tree and 162 from one cave), but numbers have also been taken in large underground drains, among old iron and concrete ruins, in rooms of abandoned dilapidated houses, in empty garrets, under bridges, in unprotected cellars under buildings and on one occasion in a disused well 30 feet deep. Some of these sleeping places have been located

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DESMODUS RUFUS IN CAPTIVITY.

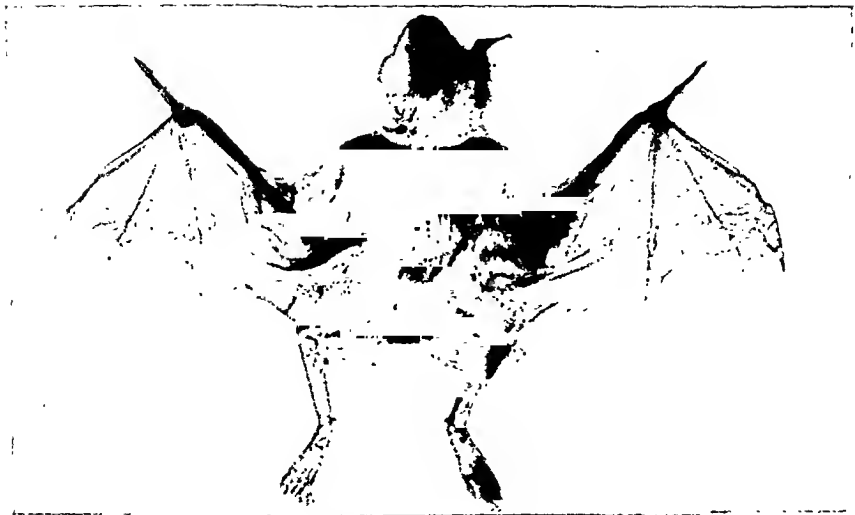
FIG. 3.—Approaching his daily blood meal as an expert walker.

FIG. 4.—Lapping his daily meal from a glass container.

FIG. 5.—Fully gorged after a good meal.

Photographs kindly lent by R. K. Ditmars.

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FIG. 6.—Young *Desmodus rufus* 5 days old ; weight 5 grammes. Note soft brown hair covering most of head and body.

FIG. 7.—*Desmodus rufus* lapping blood from a goat. Note characteristic attitude of bat.



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FIG. 8.—Window of an old abandoned house. Showing characteristic black tarry droppings of *Desmodus rufus*.

FIG. 9.—Native schoolboy—age 14—who was bitten—alternately with his pig—during a period of 2 years. Scars were visible over his toes, feet, legs, knees, fingers, forearms, elbows, back, face and forehead. He was proud of the fact and glad to be able to share the honours with his pig. His brother and mother living in the same hut were not bitten.



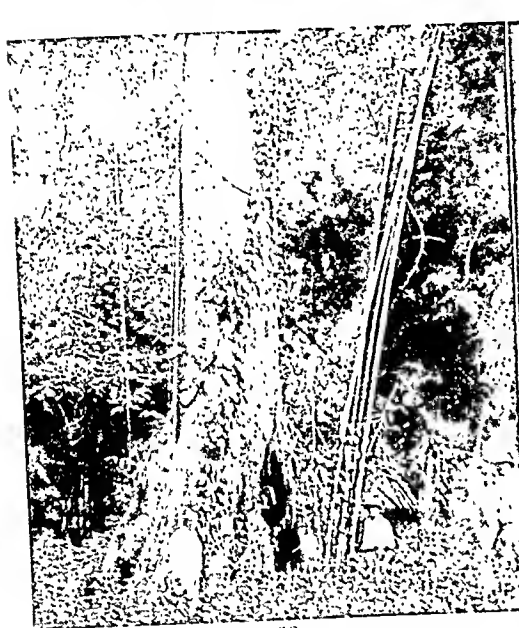
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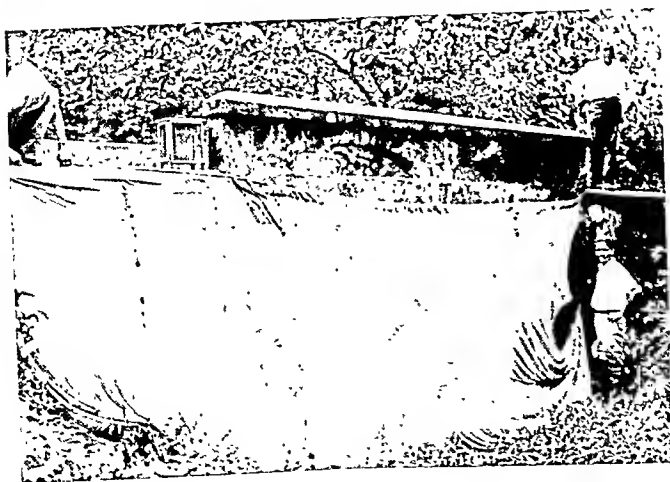
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CAPTURE AND
DESTRUCTION
OF
Desmodus
BATS.

in the immediate vicinity of thickly populated areas near Port-of-Spain and some actually in the city. (Figs. 10-13.)

When at rest, like all bats, *Desmodus* bats hang head downwards in clusters but contrary to the usual habit of bats, when first disturbed they have a peculiar way of grinning at the intruder, and walking away leisurely and often backwards into some safe dark crevice or corner in mouse-like fashion. They usually do not attempt to fly unless actually driven away by continual worrying.

Desmodus in Captivity.

When we first started our investigations, we were faced with the problem of keeping bats in confinement for experiments and observation. They were kept at first in screened houses, 10 ft. \times 9 ft. \times 8 ft., and fed on fowls and goats, on which they appeared to thrive; but as a result of a communication to us by Dr. R. L. DITMARS (Curator of the Department of Mammals and Reptiles, New York Zoological Park) of his experience in New York with *Desmodus* bats obtained from Panama, the keeping of these bats in small cages, 12 in. \times 12 in. \times 9 in., was greatly facilitated by the use of defibrinated blood in saucers. This method of feeding was first practised by L. H. DUNN of Panama. Up to date some of these bats have been kept in captivity in this way for over nine months and baby bats have been born in the cages and are doing well. Live consignments of these bats have recently been sent with success to the New York Zoological Park and London Zoological Gardens by Professor URICH from Trinidad by using frozen defibrinated blood; and a recent newspaper report states that a group of twenty-four vampire bats has been sent alive from Costa Rica to Buenos Ayres by using defibrinated blood. (Figs. 3, 4 and 5.)

ASSOCIATION OF OTHER BATS WITH *Desmodus*.

Although as a rule communities of bats keep to themselves, several other species have been found associated with *Desmodus*, especially when the sleeping

FIG. 10.—Opening of old underground brick drain, 5 feet high, 3 feet wide and 70 feet long. Disused for about 40 years. About 150 *Desmodus* bats have been caught in this drain.

FIG. 11.—Ruins of an old water wheel. About 60 *Desmodus* bats were caught here near the ground in the spaces between the paddles on the outer rim of the wheel.

FIG. 12.—First chamber and entrance to the dark second and third chambers of a small cave where about 50 *Desmodus*, and numerous *Hemiderma* (fruit-eating) bats were destroyed.

FIG. 13.—Hollow tree near the Caroni River (tidal). 30 *Desmodus* and 80 *Noctilio* (fish-eating) bats were caught in this hollow tree while several insectivorous bats lived outside near the entrance.

FIG. 14.—Capturing live *Desmodus* bats in a hollow hog plum tree. After netting the hollow space, the small boy pokes the bats down with a long rod, and the bats are caught as they dive down and try to escape at ground level.

FIG. 15.—Netting of *Desmodus* bats whilst digesting their meal under a culvert, usually between 8 and 11 p.m.

places are large. The following species have been captured in sleeping or digesting places of *Desmodus* :—*Hemiderma brevicauda* and *Artibeus planirostris*, fruit eaters ; *Noctilio*, a fish eater ; *Vampyrus spectrum*, a flesh eater ; *Enchisthenes harti*, *Saccopteryx* and *Micronycteris megalotis*, insect eaters ; and *Phyllostoma hastatum*, a mixed flesh and fruit eater.

ECTOPARASITES OF BATS.

The study of the ectoparasites of bats is of special importance at present owing to the recent discovery by the Panama authorities of the susceptibility of *Desmodus* to trypanosome infection and, therefore, to the possibility of the spread of this disease through the agency of such parasites. Although this method of infection is not considered likely in the case of paralytic rabies, the study of such parasites is nevertheless not being overlooked. Up to date only two species of parasite have been found by Professor URICH. Streblid flies are very commonly seen on *Desmodus* and on all the other bats found associated with it, and in some instances acarids have also been observed.

V. BAT DESTRUCTION WITH SPECIAL REFERENCE TO METHODS OF DESTRUCTION AND CAPTURE OF *Desmodus rufus*.

Desmodus rufus.

Desmodus bats may be destroyed or captured as follows :—(A) on their feeding grounds ; (B) in their digesting places ; and (C) in their roosting places.

(A) 1.—As *Desmodus* bats have the habit of biting the same animals on the same spots nightly, and usually bite a comparatively small proportion of animals herded together—one bite apparently serving the need of a large number of bats night after night—poisoning, by means of a local application of strychnine to such animal bites, has proved to be an eminently practical method of destroying these bats. The use of strychnine in poisoning fruit-eating bats (*Hemiderma*) had previously been successfully used by Professor URICH by placing a small amount of strychnine in a ripe banana. The application of this method of poisoning to *Desmodus* was first demonstrated by M. D. LUMSDEN, B.V.Sc., of the Trinidad Department of Agriculture. Strychnine made into a thick paste with syrup (30 grains to 1 ounce) is painted by a small brush on the wounds of the bitten animals about nightfall and some of the poisoned bats may be collected next morning in the immediate vicinity of their victims, while others are found some distance away. The largest number found poisoned from one wound was five. This method of destruction is now being put to practical use on a large scale with excellent results.

The use of arsenite of soda and tartar emetic on similar lines is also being given a trial.

In the experiment previously mentioned, where 200 animals were observed for 4 months, the average number of animals bitten was 18 before applying the poison, and this was reduced to 4 after applying it.

2.—Small portable trapping cages with fowls and animals as bait have also been successful, but have only a limited use.

(B).—Early in the campaign, before the poisoning method described above was known, a fair number of *Desmodus* bats were trapped at night between 8 and 11 p.m. under bridges by netting the bridge and subsequently capturing the bats with hand nets. This proved to be an effective method, but it had its limitations owing to the night work involved and the difficulties encountered during periods of rain. The largest number caught at a time under any one bridge was eleven. A fair number of these bats have also been shot in animal sheds while digesting their meal. (Fig. 15.)

(C).—From experience gained during recent months, the most effective and satisfactory method of controlling the *Desmodus* population is by destroying them in their sleeping places during the daytime. The actual method of destruction must vary according to circumstances, but netting and shooting usually play an important part, whilst in the case of certain hollow trees and underground drains and certain other suitable localities poisoning by Cyanogas A dust is very successful. Often the bats are able to seclude themselves in the long tortuous fissures and crevices and in the small and narrow tunnels and chambers of the caves, as well as in the upper portions of hollow trees. In such cases resort is usually had to fumigation by burning sulphur or dried green leaves. It is astounding what an amount of SO_2 and smoke the bats will stand before they decide to fly out into the nets which are set for them outside. Occasionally, too, the dangerous nature of the caves does not permit of any work inside. In such instances all the exits from the cave are blocked except one, the mouth of which is securely netted at about nightfall, and the usual preparations made for the capture of the bats with hand nets, clubs, cages, etc. The bats usually fly out in small groups from about 7 to 10 p.m. and are readily caught. Complete blocking of all the exits of the cave, where possible, would of course also end in their starvation. (Figs. 14 and 15.)

Bats other than Desmodus.

The only other bats which appear to have a special interest for us in connection with paralytic rabies at present are the two fruit-eating bats, *Artibeus* and *Hemiderma*, both of which have been found naturally infected with the virus.

Both of these bats are found in the towns and in the country, but *Artibeus* is essentially a town bat and *Hemiderma* a country bat. They are commonly seen in the island and may be classed as agricultural pests. *Artibeus* has semi-diurnal habits and sleeps under the eaves of houses, the branches of trees and the fronds of palms. *Hemiderma* is the commonest bat in the island and has a large variety of sleeping places and is very commonly found associated with *Desmodus*. Numbers of these bats are being destroyed during the present campaign, usually by means of various netting devices with the main object of having their brains examined for Negri bodies.

The following tables show the number of all bats destroyed at their feeding, digesting and sleeping places :—

TABLE II.
DESTRUCTION OF *Desmodus rufus* BATS IN CONNECTION WITH ANTI-RABIC CONTROL MEASURES.

Year.	Month.	Feeding Places.				Digesting Places.				Sleeping Places.								Grand Total.
		Poisoned.	Trapped.	Killed.	Total.	Under Bridges.	Stables.	Uninhabited Houses.	Total.	Hollow Trees.	Caves.	Underground Drains and Tunnels.	Old Iron and Concrete Ruins.	Dry Disused Wells.	Uninhabited Houses.	Dark Cellars.	Total.	
1934	Feb. to Dec.	0	9	3	12	57	0	0	57	39	7	46	0	0	5	0	97	166
1935	Jan. to May	149	0	8	157	29	0	0	29	204	9	3	0	15	0	16	247	433
1935	June and July	100	0	5	105	0	0	0	0	96	428	153	67	0	48	17	809	914
1935	Aug. and Sept.	21	12	0	33	11	0	0	11	310	398	269	1	0	16	0	994	1,038
Grand totals (1934-1935)		170	21	16	307	97	0	0	97	649	842	471	68	15	69	33	2,147	2,551

TABLE III.

DESTRUCTION OF THE FRUIT-EATERS *Artibeus* AND *Hemiderma* IN CONNECTION WITH ANTI-RABIC CONTROL MEASURES.

Year.	Month.	Fruit Eating Bat	Feeding Places.			Digesting and Sleeping Places.										Grand Total.	
			Fruit Trees.	Houses.	Total.	Hollow Trees.	Caves.	Under Bridges.	Underground Drains and Tunnels.	Galleries and Unfrequented Rooms.	Disused Wells.	Old Iron and Concrete Ruins.	Palms and Other Trees.	Eaves of Houses.	Garrets.		Total.
1934	Feb. to Dec.	<i>Artibeus</i>	0	0	0	0	7	0	0	0	0	0	0	44	0	51	51
1935	Jan. to July	"	0	1	1	0	0	0	0	10	0	0	106	217	0	334	335
	Aug. and Sept.	"	0	0	0	0	26	0	0	0	0	0	140	195	0	361	361
Grand total (1934-1935)			0	1	1	0	33	0	0	10	0	0	246	456	0	746	747
1934	Feb. to Dec.	<i>Hemiderma brevicauda</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
1935	Jan. to July	"	0	6	6	19	45	7	0	3	1	0	0	0	0	75	81
	Aug. and Sept.	"	0	0	0	0	365	0	3	67	8	0	0	6	2	451	451
Grand total (1934-1935)			0	6	6	19	410	8	3	70	9	0	0	6	2	527	533

N.B.—These bats were caught with the main object of having their brains examined for Negri bodies.

TABLE IV.

DESTRUCTION OF BATS (OTHER THAN *Desmodus*, *Artibeus* AND *Hemiderma*) IN CONNECTION WITH ANTI-RABIC CONTROL MEASURES.

	Number destroyed.
1934, February to December	100
1935, January to July	154
„ August and September	86
Total	340

N.B.—These Bats were caught with the main object of having their brains examined for Negri bodies.

VI. LABORATORY EXPERIMENTS AND RESULTS OF EXAMINATION OF BATS' BRAINS FOR NEGRI BODIES.

LABORATORY EXPERIMENTS.

The following laboratory experiments have so far been reported from Trinidad and from Brazil.

Trinidad.

In addition to the work by HURST and PAWAN previously mentioned, Dr. J. L. PAWAN (see References), Government Bacteriologist, has reported :—

1. That an emulsion of the brain of a naturally infected *Desmodus* bat caught while biting an animal, when injected subcutaneously into a calf, was able to produce paralytic rabies in that calf.

2. That the virus of a naturally infected bat from an infected area was successfully inoculated into a monkey (species not reported) and then passed on through a series of calves, producing typical symptoms of the disease as it occurs in the field under natural conditions.

3. That the inoculation of dogs with virus from field cases direct has always proved negative, but that after passage through monkeys it was possible to produce the disease in four dogs. In no case, however, did the dogs show symptoms of furious rabies, the dumb form with paralysis of the hind legs and jaw being always constant.

4. That the saliva from both human and animal cases is infective and is able to transmit the disease to rabbits.

Captain H. V. METIVIER (see References), Government Veterinary Surgeon, has also reported the following experiments :—

1. In October, 1931, shortly after the publication of HURST and PAWAN's paper three *Artibeus* bats (fruit-eaters) were caught at the Government Farm whilst biting and fighting with each other. An emulsion made of the brains and salivary glands of these three bats, when inoculated subcutaneously into a guineapig, a rabbit and a calf, produced typical symptoms of paralytic rabies in each animal. These were the first bats proved to be infected with rabies in Trinidad.

2. The inoculation of dogs with virus from field cases direct has always proved negative. The experiments carried out at the Government Farm confirm Dr. PAWAN's experiments at the Government Laboratory.

3. The inoculation of milk from an infected cow into the cheek muscle of a calf proved negative.

4. Guineapigs remained quite healthy after being inoculated with macerated ticks which had been fully gorged with blood from an infected animal.

5. Two *Desmodus* bats injected with brain emulsion from an infected animal did not contract the disease.

6. Three *Desmodus* bats, after having infected animal salivary glands rubbed into their scarified skin, did not contract the disease.

7. Three *Desmodus* bats inoculated intramuscularly and subcutaneously with blood from an infected animal failed to contract the disease.

8. Two fruit-eating bats, after having infected animal salivary glands rubbed into their scarified skin, also failed to contract the disease.

N.B.—All these bats remained alive for several months after the experiments and before being examined for Negri bodies.

Brazil.

A number of workers at the Institute of Animal Biology (1934) have shown that :—

1. Neither the dog nor the small carnivorous animals belonging to the dog and cat families were in any way affected by the epizootics.

2. That a live *Desmodus* bat caught biting a cow in an epizootic focus was proved to have the virus of rabies in its salivary glands and brain.

3. The field hart, the tapir and the monkey are subject to the disease after inoculation, but cannot ordinarily be the agents of transmission.

4. Direct contagion among infected white mice, guineapigs and cattle was not possible.

5. Bullocks fed with hay moistened with saliva from infected cattle did not contract the disease.

6. Bullocks bitten by flies and ticks feeding on infected cattle failed to contract the disease.

7. The saliva from infected cattle failed to infect white mice and guineapigs. [N.B.—Dr. PAWAN proved it infectious to rabbits.]

8. *Desmodus* cannot be infected by lapping blood from infected cattle.

9. A *Desmodus* bat inoculated with bovine rabies can infect healthy bats when kept in confinement with them.

10. Four *Desmodus* bats after inoculation with bovine rabies virus, though showing no symptoms of rabies themselves, were capable of transmitting the infection to twenty-five healthy cattle within 1 to 4 months after experimental inoculation. One such bat killed after 5 months was proved to have its salivary glands infected.

Confirmation of this last experiment would mean not only a new addition to our knowledge of the virus of rabies, but would also further indicate the necessity for considering the carrier problem in connection with viruses in general.

In addition to these reported experiments a number of experiments are now in progress in Trinidad dealing with *Desmodus*, *Artibeus*, *Hemiderma* and other species of bats. These should prove to be of definite value in working out the epidemiology of the disease and possibly in instituting more effective control measures.

It should be noted that the diagnosis of rabies both in Trinidad and Brazil has been made by (a) its symptoms, (b) experimental inoculation, (c) the presence of Negri bodies and (d) the power of immunity to the disease given by the vaccine prepared from the fixed virus.

EXAMINATION OF BATS' BRAINS FOR NEGRI BODIES.

Towards the end of 1934, as the various methods of capture and destruction of *Desmodus* were gradually being devised, it was realised that the routine examination of the brains of bats for the presence of Negri bodies, which is an accepted proof of the presence of rabies, would be of material assistance in locating the foci of infection in the bats, not only in known infected areas where animal and human cases of paralytic rabies had occurred, but also in non-infected areas ahead of the endemic and epidemic centres.

Table V (on opposite page) shows the results of these examinations.

The highest percentage of infection in *Desmodus* bats was obtained in the following roosting places. Most of the other infected *Desmodus* were found as isolated cases :—

TABLE VI.

Roosting Place.	Number of <i>Desmodus</i> Captured.	Number Examined.	Positive.	Per cent. Positive.
Casteel Cave	116	111	16	14.4
Arranguez, underground drain	166	161	19	11.8
Valencia, 2 hollow trees ...	47	37	3	8.1
Maraval Cave	37	36	3	8.3

VII. EPIDEMIOLOGY AND CONTROL OF PARALYTIC RABIES.

EPIDEMIOLOGY.

The following summarized factors appear to have a definite bearing on the epidemiology of the disease :—

1. Paralytic rabies exists naturally in Trinidad among animals and human beings. The disease is easily recognised by those who have seen cases previously. The affected animals include cattle, horses, mules, donkeys, pigs, goats and sheep.

2. Both the animal and human diseases occur in epidemic form in definite groups over comparatively short periods of from 1 to 10 weeks, after which it appears to linger in endemic form in the affected district as evidenced by the occurrence of a number of more or less isolated cases.

TABLE V.
RESULTS OF HISTOLOGICAL EXAMINATION OF BATS' BRAINS FOR PRESENCE OF NEGRI BODIES.*

Year.	Month of Capture.	Bat.	Infected Areas i.e. Where Animal or Human Cases have recently occurred.				Non-Infected Areas, Bordering on Infected Areas.			
			Number Examined.	Positive.	Negative.	Per cent. Positive.	Number Examined.	Positive.	Negative.	Per cent. Positive.
1934	May	<i>Desmodus rufus</i>	2	1	1	50	0			
1935	Oct.-Dec.	"	49	7	42	14.3	13	1	12	8
	Jan.-May	"	131	3	128	2.3	96	2	94	2.1
	June and	"	384	42	342	11	101	1	100	1
	July	"	566	53	513	9.4	210	4	206	1.9
1934	Oct.-Dec.	<i>Artibeus</i>	12	1	11	8.3	7	0	7	0
1935	Jan.-July	"	22	0	22	0	68	0	68	0
			34	1	33	3	75	0	75	0
1935	Jan.-July	<i>Hemiderma</i>	41	1	40	2.4	8	0	8	0
1934	Oct., 1934	<i>Phyllostoma</i>								
1935	to	<i>Noctilio</i>								
	July, 1935	<i>Vampyrus spectrum</i> <i>Glossophaga</i>	19	0	19	0	19	0	19	0

* This examination was kindly undertaken by Dr. J. L. PAWAN, Government Bacteriologist.
Heavy type = Totals.

3. In a certain number of the endemic areas, the disease has been known to flare up again in more or less epidemic form at a later date.
4. The disease, once established in a district, remains there for a period varying from about 6 to 18 months or 2 years or even longer, after which it dies out completely and does not return for several years.
5. In the case of the 1935 outbreak this period of complete freedom from the disease had lasted 6 years.
6. There appears to be a definite seasonal fluctuation in the incidence of the disease except perhaps in 1935.
7. The first evidence of the existence of the disease among the bats consists in an increase in the frequency of biting of animals and persons. The animal disease has also invariably preceded the human disease. The mortality in both cases is 100 per cent.
8. Though the animal disease has been known to occur in fairly large numbers over the whole island at one time or another from 1925 to 1935, the human disease has remained confined to comparatively few areas only and altogether 55 cases have occurred between 1929 and 1935.
9. The bat is the only other mammal found by Dr. PAWAN to be naturally infected in Trinidad.
10. Three species of bats have so far been found infected.
11. Two of the species are fruit eaters and presumably, therefore, incapable under ordinary conditions of transmitting the disease to animals or man, but it is possible that they may act both as vectors of the disease among their own species or as reservoirs of the disease for other species of bats and especially for *Desmodus*.
12. The third bat of the species *Desmodus*, is an animal which is not only able to bite like the dog, but even after biting is apparently able to continue infecting the wound for about 15 to 25 minutes whilst lapping up the blood—the resulting dose of virus must presumably, therefore, be a very heavy one, and it is suggested that this may account for the paralytic type assumed by the disease as usually occurs after extensive bites, *e.g.* by rabid wolves (HURST and PAWAN, 1931, p. 10). With one exception a history of bat-bite has been obtained in the forty cases of human paralytic rabies investigated.
13. The great majority of the cases of paralytic rabies among human beings occurred after one bat-bite only. The incidence of cases among unprotected individuals who were bitten in two recently infected areas equalled 31 and 41 per cent. These figures are considered to be only approximately correct.
14. The incubation period in men and animals is usually about 3 to 4 weeks but may be prolonged occasionally to several months.
15. In the recent outbreaks *Desmodus* infection was found to be closely

correlated to the incidence of paralytic rabies in animals and man, and where there were a large number of cases the infection amongst the captured *Desmodus* in the immediate vicinity was correspondingly heavy.

16. *Desmodus* bats with sleeping places in or near populated centres may almost be considered as domesticated animals, and in many instances they live in very close proximity to man. Their similarity to rats in this respect is very striking.

17. Cave-inhabiting *Desmodus* with sleeping places away from populated centres appear to fly at least about 1 mile to their victims, usually down in the valley below.

18. These cave-dwelling *Desmodus* appear to live in large rather than small communities, with the result that only a small percentage of favourable caves examined are now being utilized by these bats as sleeping places.

19. Caves, hollow trees, drains or other sleeping places, when once located, continue to form excellent traps for other *Desmodus* living in that neighbourhood. It is clear that *Desmodus* bats usually have more than one sleeping place.

20. Reported laboratory experiments from Trinidad and Brazil prove conclusively that paralytic rabies now exists in Trinidad among animals, bats and human beings; and in Brazil among animals and bats. They show, furthermore, that an infected blood-lapping vampire bat is capable under natural conditions of transmitting the disease to animals and men, as well as to its own kind.

METHOD AND SPREAD OF THE DISEASE AMONGST ANIMALS, HUMAN BEINGS AND BATS.

Animals.

As *Desmodus* bats are in the habit of feeding at least once nightly on animals, it is easy to see how the introduction of one or more infected *Desmodus* into a community of bats may account for the existence of the animal disease throughout the year in a district in endemic or epidemic form.

Human Beings.

In the case of human beings, however, the following two epidemiological findings appear to require some explanation:—I. Why is the disease in human beings confined only to certain small areas, whilst it is actually prevalent in animals over very large areas? II. What is the actual cause of the outbreak of an epidemic and why is it seasonal? (This question, of course, also refers to animals.)

I. As an explanation for the limitation of the human disease to certain small areas only, such reasons as the following might be put forward : (1) The bat-proofing of animal sheds in such areas ; (2) the removal of animals from such areas ; (3) habitual biting of certain persons by bats in those areas. From field experience the above three explanations, though operating in some instances, do not appear to hold good as a whole ; (4) a fourth explanation is now suggested, *viz.* " A change of habit in the infected bat at some period of its infected life." As offering some evidence of this possibility two *Desmodus* bats, among a large number of others kept under observation in cages, were seen to be restless and unusually active in the daytime and died within a few days. On examination of their brains, Negri bodies were found. Is it possible that these bats might be the " daylight " bats previously mentioned and that these " daylight " and changed habits appear only a few days before death ; and, further, that a few of these bats operating under favourable conditions, as mentioned in (1), (2) and (3) above, may prove to be the main cause of the human element of the disease ?

II. The explanation for the cause of an outbreak of an epidemic among animals and men, which usually occurs about the same time, might also lie in the same direction, as presumably a community of infected bats might well provide a sufficient number of such bats with changed habits operating at the same time.

With regard to seasonal fluctuation in the incidence of the disease the possible increased seasonal breeding of the bats at certain periods would appear to offer the most reasonable explanation, though there is as yet no definite evidence of this.

Bats.

As to the method of spread of the disease among *Desmodus* bats, it would appear reasonably certain that it is possible for one or more infected *Desmodus* (roosting in clusters as they do in such close and intimate contact with the other members of a community) to cause the gradual infection of the whole or the greater portion of that colony over a period of several months. As evidence of this, it has been found that a fair percentage of *Desmodus* bats, when captured, show numbers of scars over their heads and bodies, and also that *Desmodus* bats, when kept hungry in confinement together, commonly attack each other for feeding purposes.

It is also suggested that, while feeding from the same wound in the same animals, healthy *Desmodus* from neighbouring colonies might easily become infected by *Desmodus* from an infected colony, either through biting or from infected saliva *via* the mucosa of the mouth or intestines.

In this manner, the disease might presumably spread from district to district, and it is inferred that the several colonies of bats responsible for the human or animal outbreak in a locality, might automatically and gradually become exterminated over a period of 6 to 18 months or 2 years, the duration of the outbreak

being dependent on the number and size of the colonies responsible for the outbreak and on the proximity of their various feeding grounds.

The infection among the fruit-eating bats would appear to have originated from the bites of infected *Desmodus* and to be maintained similarly, as well as through the infected bites of their own species. It is also possible that fruit-eating bats, when rabid, might attack and infect *Desmodus* bats of their own as well as of neighbouring districts as their range of flight is more extended owing to the enforced necessity of searching out fruit-bearing trees over a wide area during the various seasons. This possibility should also be borne in mind in connection with the seasonal fluctuations of the disease.

CONTROL OF THE DISEASE.

If the probable method of spread above described be accepted as a working basis, the control measures in order of importance would appear to be as follows :—

1. Destruction of all *Desmodus* bats in all areas where paralytic rabies is actually occurring in animals or human beings: the complete absence of bat-bites amongst animals, fowls or man to be the final test of success.

2. Destruction of individuals and colonies of *Desmodus*, *Artibeus* and *Hemiderma* for purposes of brain examination for the presence of Negri bodies in all non-infected areas in close proximity to infected areas as at (1). Control measures as at (1) should be immediately instituted in all such areas where infected bats are found—the prevention of the spread of rabies from the bat to animals and man in such areas to be the final test of a successful campaign.

3. Protective inoculation and protection from bat-bites.

4. Educational and propaganda work for encouraging co-operation in control measures. In this connection, a film embodying the results of the above work has been prepared locally by the authors and produced by the Tucker Picture Co. of Trinidad. This is proving to be of some definite educational value.

5. As the locating of *Desmodus* sleeping places is sometimes difficult and might entail dangerous delay, a complete survey to show and record all known and possible feeding, digesting, and sleeping places throughout the island is now in course of preparation.

The following five charts (Figs. 16-20) deal with surveys which were carried out during the two local outbreaks of twenty cases from April to July, 1935, and show clearly the epidemiological findings and the method of spread and control of the disease as outlined above.

Three of the surveys deal with infected areas, *i.e.*, areas where animal or human cases were occurring; whilst the fourth shows a non-infected area bordering on two of the above infected areas; the fifth deals with the City of Port-of-Spain, also a non-infected area in close contact with two of the above infected areas.

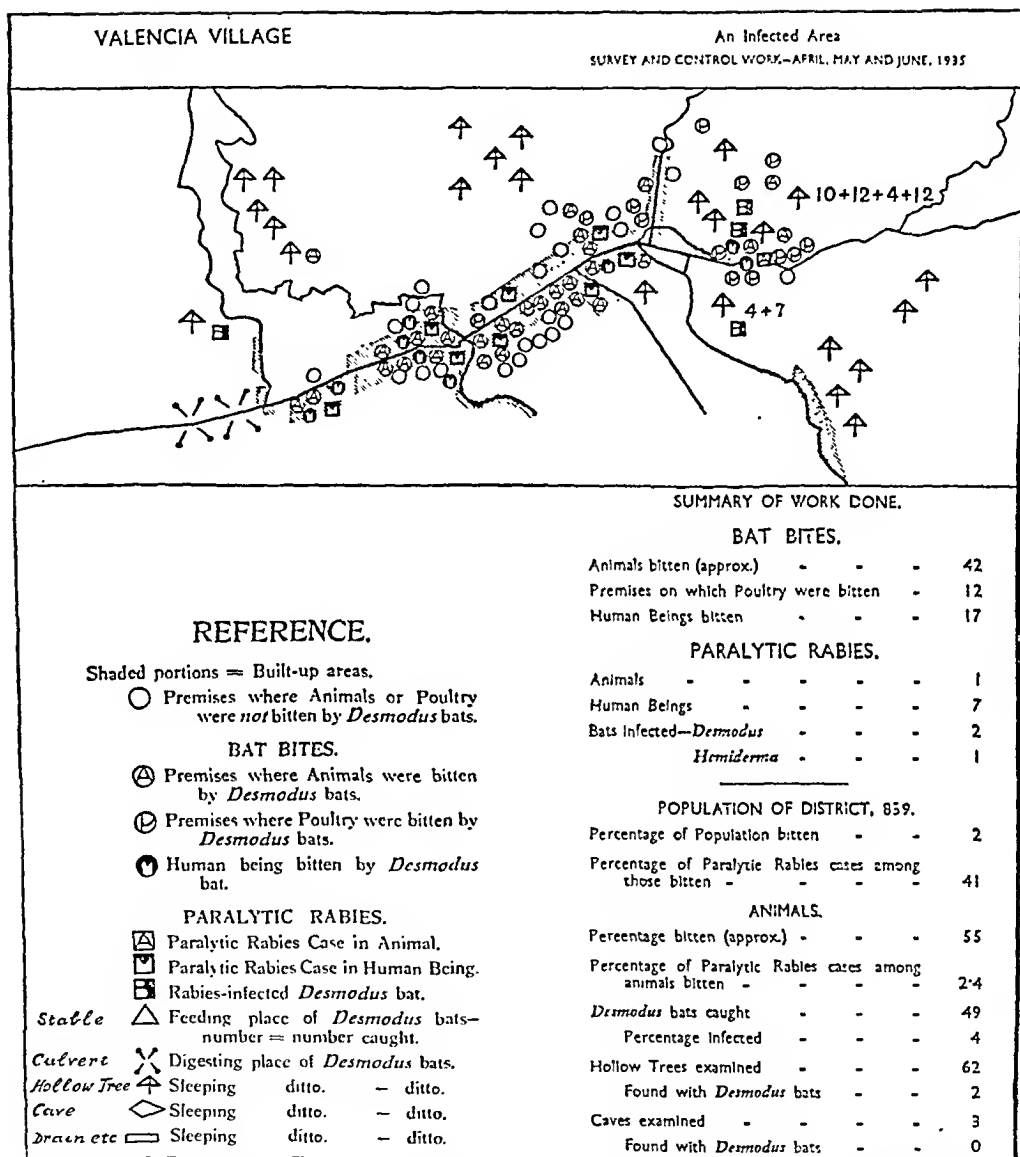


Fig. 16.—VALENCIA. This is a small recently established village surrounded by forest about 24 miles from Port-of-Spain.

Note: (a) The low incidence of animal paralytic rabies due to intensive anti-rabic vaccination some months previously.
(b) The high incidence of the human disease among persons bitten.
(c) The small weekly capture of *Desmodus* bats from only 2 of 62 hollow trees examined.

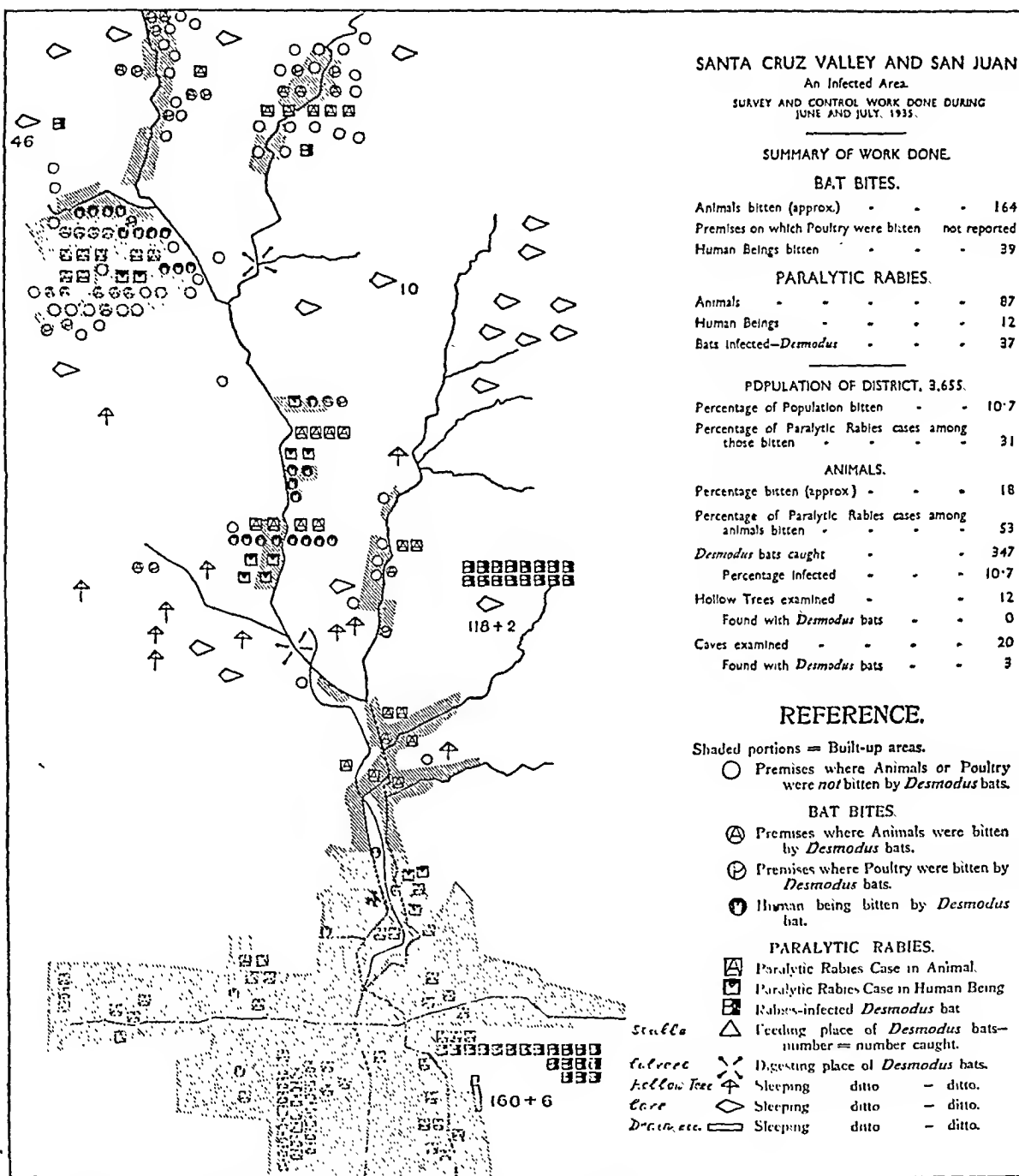


Fig. 17.—SAN JUAN VILLAGE AND SANTA CRUZ VALLEY. San Juan is an open, thickly-populated dairying village 3 to 4 miles east of Port-of-Spain. Santa Cruz Valley extends northwards and consists of a number of cocoa estates surrounded by forest-clad cave-bearing hills about 500 to 1,000 feet high.

Note: (a) The high incidence of animal paralytic rabies—no recent vaccination had been undertaken here.
(b) The high incidence of the human disease among persons bitten.
(c) The high incidence of the disease among the *Desmodus* bats captured in the Casti Cave 1 mile above the valley as well as in the Aranguéz underground drain at San Juan.
(d) The close correlation existing between the animal and human diseases and the rabies infection amongst the *Desmodus* population.

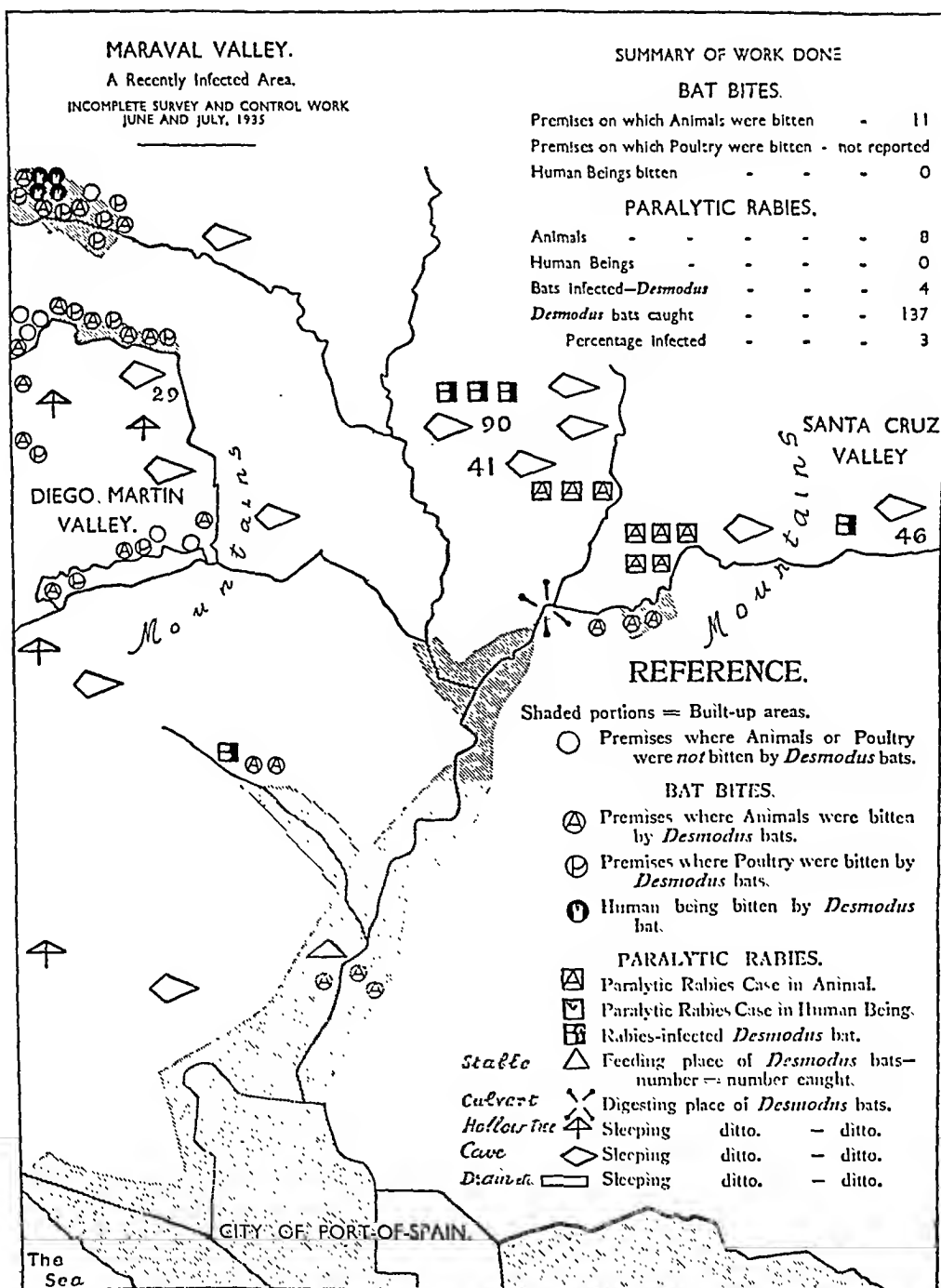


Fig. 18.—MARAVÁL. This narrow residential and cocoa cultivated valley lies about 4 miles north of Port-of-Spain and is surrounded by cave-bearing hills about 1,000 to 1,500 feet high.

Note: (a) Its proximity to the neighbouring infected Santa Cruz Valley.

(b) The incompleteness of the survey.

(c) The early correlation between the animal disease and the infection in the bats.

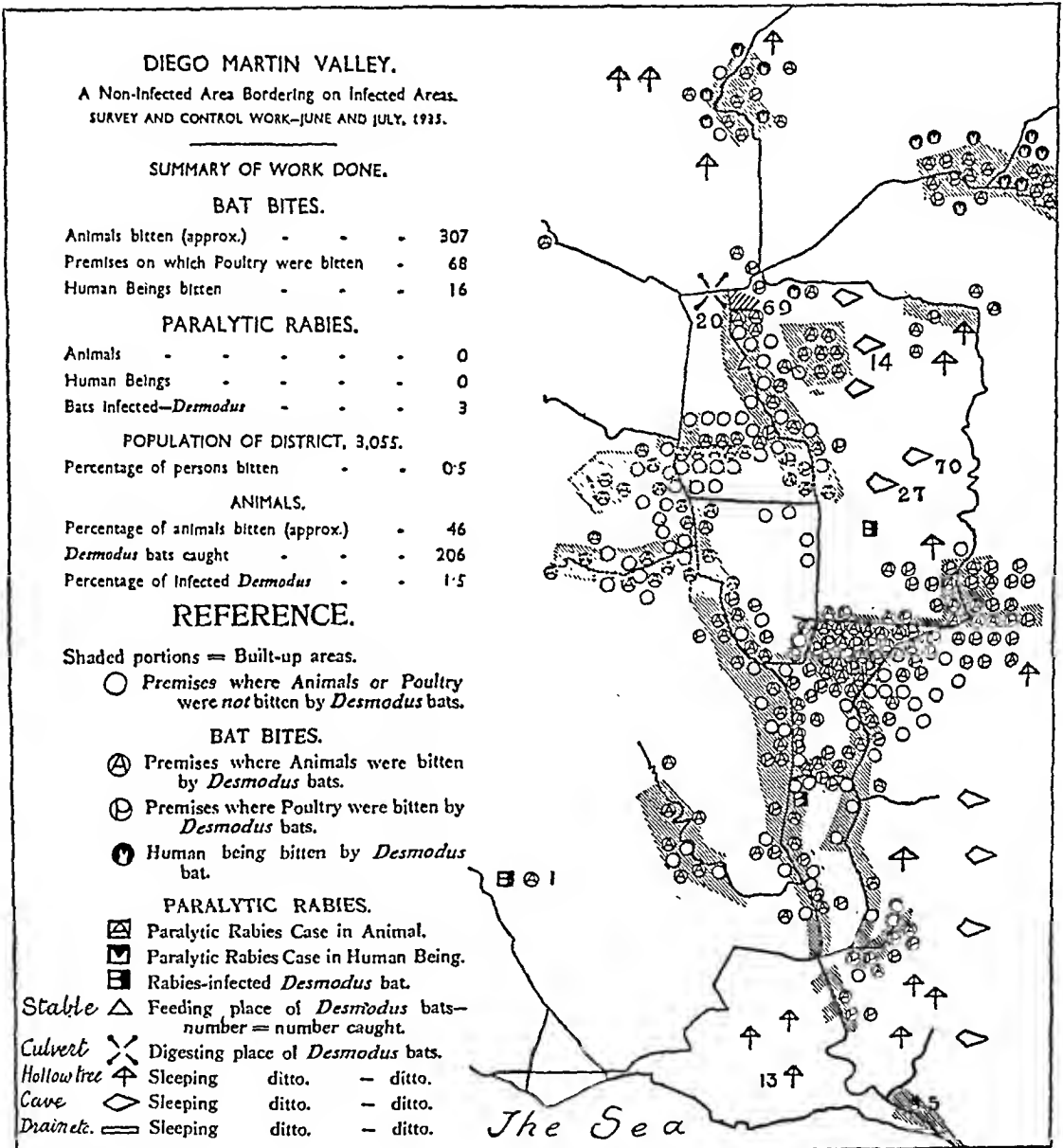


Fig. 19.—DIEGO MARTIN. This is an old-established populated cocoa-growing area 4 to 8 miles west of Port-of-Spain. The hills around are all cave-bearing and forest-clad, in parts, and about 1,000 to 1,500 feet high.

- Note: (a) The absence of animal or human paralytic rabies cases.
 (b) The presence of three infected *Desmodus* bats.
 (c) The intensive biting of only animals and poultry in the southern section.
 (d) The high incidence of biting of human beings in the northern portion.
 (e) Its proximity to the neighbouring infected Maraval Valley on the East.

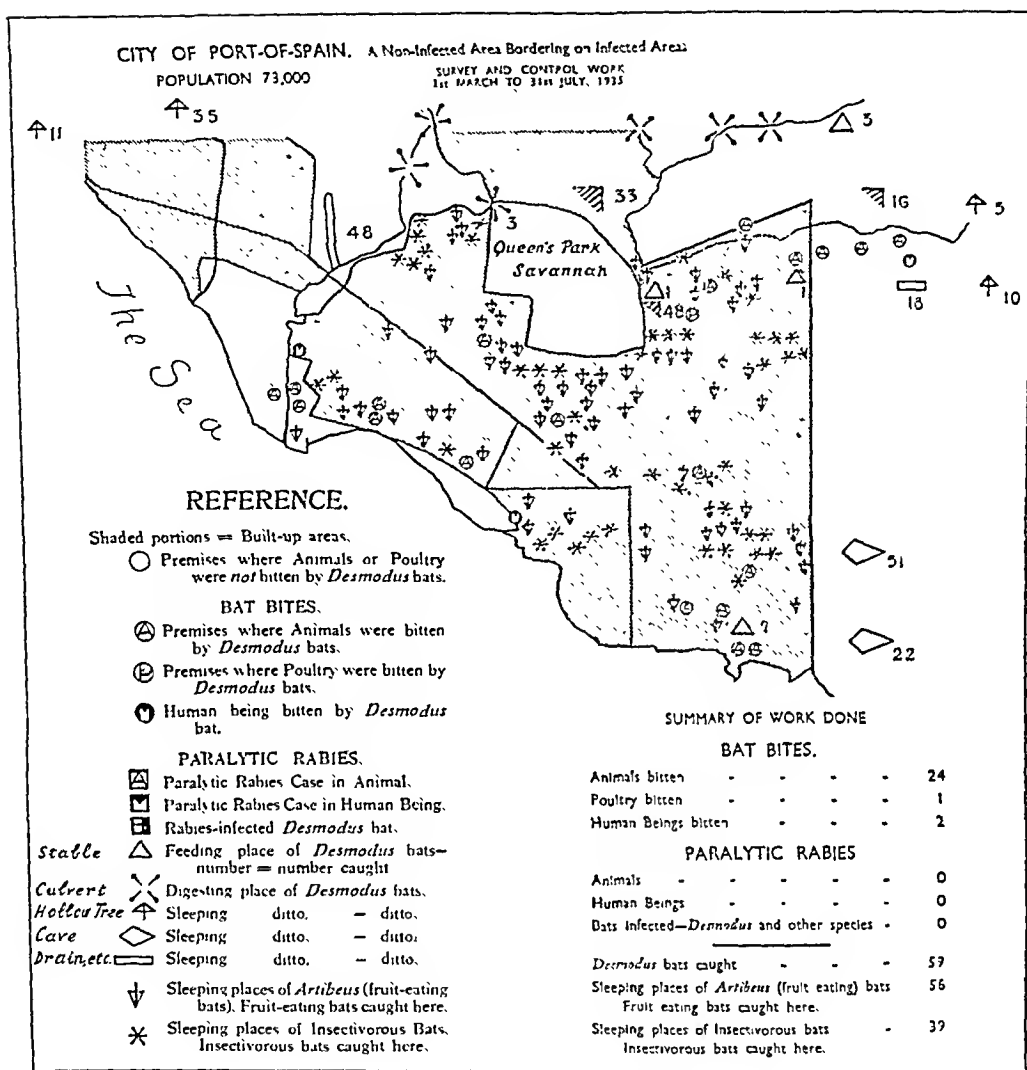


Fig. 20.—PORT-OF-SPAIN. This is the capital of the island, situated on the Gulf of Paria about 25 miles from Venezuela and is surrounded by cave-bearing hills about 200 to 800 feet high. Population about 73,000.

Note: (a) The complete absence of animal or human paralytic rabies as well as of infected *Desmodus* bats.

(b) The low incidence of bat-bites especially among human beings in spite of

(c) The comparatively large number of *Desmodus* caught in the immediate neighbourhood as well as in the town.

(e) The large number of fruit-eating and insectivorous bats being destroyed in the town as a further safeguard.

VIII. CONCLUSION.

The following conclusions would appear to be justifiable :—

1. Paralytic rabies exists on a fairly large scale amongst animals in Brazil and in Trinidad. The human form of the disease has so far been reported only from Trinidad, and to a limited extent only.

2. We must consider the possibility that the disease may be due to a modified form of the virus of rabies. The vampire bat *Desmodus* is the only known vector.

3. The control of the disease is dependent principally on the control of the *Desmodus* population in the affected areas, but the possible relation of fruit-eating and other bats to the spread of the disease appears to require further investigation.

4. Effective methods of destruction of *Desmodus* and other bats have been evolved in Trinidad and should prove to be of value elsewhere.

5. In view of the fact that the proved transmitter of the disease exists throughout South and Central America and is a flying mammal with an apparently fairly wide range of flight, the disease with a mortality rate of 100 per cent. should be considered as a serious public health problem, not only to Brazil and Trinidad, but also to the rest of South America and Central America.

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DISCUSSION.

Dr. J. W. Lindsay : What I have to speak about on the present occasion is bovine paralytic rabies. What we have been hearing from Dr. DE VERTEUIL this evening has impressed me so much that I hardly know how to begin, because I shall be taking you away back so far, to the time when we knew nothing of the subject on which he has been speaking.

I should like, first of all, to emphasise the economic importance of the subject, especially in connection with the control of paralytic rabies. Dr. DE VERTEUIL has spoken of Trinidad. We have with us tonight Dr. MAGARINOS TORRES, of the Oswaldo Cruz Institute, Brazil, and I feel rather shy about speaking before him, seeing that nearly all I have to say I have learned from his Institute. We have in Paraguay a national doctor who was trained in Brazil and it was he who, when he came to Paraguay, began seriously to investigate the question of bovine paralytic rabies. In South America a huge territory is involved ; and in the last 10 years hundreds of thousands of cattle have been lost in Brazil, Argentina and Paraguay, where there has appeared in recent years an epizootic disease, attacking cattle, horses and pigs, with symptoms of paralysis. In a paper published in the TRANSACTIONS in 1933 my son, Dr. KENNETH LINDSAY, wrote :—

“During recent years a new disease has made its appearance among cattle. It is known locally as bovine mal de caderas, but this is a misnomer. One searches in vain for *Trypanosoma* in both the blood and the cerebrospinal fluid of affected animals. Negri bodies are to be found in the spinal cord, and MIGONE and PEÑA (1932) and others regard the disease as a form of rabies. It is spreading slowly throughout the continent and causes great loss of stock. Usually the more robust animals go down with it first. The mode of transmission is not known, but vampire bats have been suspected. No remedy has been found, and the disease is always fatal, death usually taking place about the third day.”

In Paraguay, in September, 1928, the National Institute of Parasitology was notified of the outbreak in certain estancias or ranches of alarming epidemics of “mal de caderas” among the cattle. It was well known that cattle did die with symptoms similar to those of mal de caderas of horses, but such cases had never been investigated. When, however, in 1928 the cattle were dying at the rate of 25 to 30 per cent. the cattle owners became alarmed. The chief investigations were made on the estancias of the Liebig Extract of Meat Co., Ltd., in Paraguay. All the most modern methods for the care of cattle were in use by the Liebig Company—segregation of herds in separate paddocks, the burning of animals dead from disease, disinfectant bathing of pure bred stock, etc.

The chief symptoms of this disease, in which the finest of the stock fell sick, were loss of appetite, general indisposition, hair rough, tremors of the body, lameness and dragging of hind legs, and weakness of forelegs, with diarrhoea and sometimes (especially in milk-cows) constipation—this continues for about 3 days, after which the animal falls down and cannot rise again, dying

in another 3 days or so. Postmortem examination of dead animals showed congestion and haemorrhages of the meninges and spinal membranes, but these were not such as to have in themselves produced the paralysis, which was, therefore, considered to be caused by the intoxication of the central nervous system by the toxins of the causative agent of the disease.

The Paraguayan pathologists, since the days of their first teacher, ELMASSIAN, had been well able to diagnose mal de caderas by at once demonstrating the trypanosome in the ordinary cases of the horse disease, but in this new disease neither the repeated and methodical examination of the blood, nor experimental inoculations had been able to demonstrate the trypanosome or any other protozoon. Differential diagnosis was thus made between it and other similar diseases of a "septicaemia haemorrhagica" type.

Within the last 10 years there have been two very widespread epidemics of canine rabies, in which the dog population in many parts almost completely disappeared. There were very few cases of human rabies with hydrophobia, but very many cases of domestic animal rabies among sheep, cows, calves, pigs and cats. The last epidemic of canine rabies was in 1931, and it coincided with the subsidence of the "bovine mal de caderas" (so-called) epidemic of 1928-31; while the previous canine rabies epidemic of 1926-27 had preceded the appearance of the cattle disease.

It was at that later date (1930-31) that the Paraguayan pathologists, probably following up the suggestions made of some connection between "rabies" and the new disease, discovered what they had previously missed, *viz.* Negri bodies in the central nervous system of animals that had died from "bovine mal de caderas," or, as they had called it, "pasteuriosis paresiante del Paraguay." They also adopted the suggestion that the infection was carried by bats, and investigations were immediately made along that line as to the habits of the local bats in relation to cattle, with the result that they found that bats, caught in the epidemic areas, showed abundance of Negri bodies and must therefore be blamed as the carriers of the virus that produced the disease.

Sporadic cases continue to occur in Paraguay, although, now that its cause is assumed to have been proved, the authorities and the ranchers work in closer co-operation for its control.

Let me mention in more detail a few historical and geographical points connected with paralytic rabies (bovine) in South America. As early as 1911 there occurred in the Province of Santa Catharina in Southern Brazil a cattle epidemic that was diagnosed by the Sao Paulo authorities as a form of "rabies." KRAUS and REMLINGER and BAILLY disputed this and maintained that the disease must have been the "bulbar paralysis" of Aujeszky. Later "in 1921 HAUPT and REHAAG in Brazil reported that cattle bitten by vampire bats developed 'rabies,' and then the experimental investigation of a bat caught biting a cow confirmed the supposition that these creatures were the vectors."

In 1930-31 ROSENBUSCH was in Paraguay studying the "bovine mal de

caderas" then epidemic in the country. He sent material to REMLINGER and BAILLY and they concluded that the disease was identical with "rabies."

In Paraguay Drs. MIGONE and PEÑA of Asuncion continued the investigations. They sent material obtained from cattle dead from "bovine mal de caderas" to Professor KRAUS of Santiago, Chile, who, as the result of experiments made with the material proved that the "bovine mal de caderas" was caused by a "virus of rabies." Inoculated animals—rabbits, sheep, dogs, guineapigs—showed characteristic paralytic symptoms and died, and Negri bodies were found in their brains. MIGONE, in December, 1931, wrote, rather quaintly, that "KRAUS asks me to send him some bats for his experiments, but that for us is almost impossible." Such difficulty was indeed very real, as the great herds of cattle in the infected territories roam over widely extended areas, generally many miles from human habitations, and the bats that would infect them would live in the great forests.

At that time (1932) the Sao Paulo investigators were working to clear up some points in connection with the disease, and especially to prove that bats were indeed the transmitters of the "paralytic rabies" in cattle. There were in 1934 and there have been up to the present time, continuous investigations going on in Brazil in regard to the exact differential diagnosis between the "bulbar paralysis" of Aujeszky, and the "paralytic rabies" of bat-bite—both diseases having been proved to be co-existent in certain parts of South America. No human cases of "paralytic rabies" were reported in Brazil or Paraguay, even although it is a fact that the first cases reported in Paraguay in 1928 of the "bovine mal de caderas" were in milk cows and cart bullocks kept in the paddocks near the farms, the outbuildings of which generally harbour crowds of bats. There is no proof that they were not cases of Aujeszky's disease.

That from the economic point of view the disease must be considered as a serious menace to the tropical countries of South America can be gathered from the fact that by the year 1932, the Brazilian States of Rio Grande do Sul, Santa Catharina, Matto Grosso, Southern Amazonas and the neighbouring countries of Paraguay, Bolivia and Argentine had all been ravaged by this disease—which has thus caused enormous losses to cattle owners. Unless the disease disappears naturally from the extermination of the infected bats, the control of it will be very difficult in the outlying districts of the territories I have mentioned.

Dr. Magarinos Torres said that though he had himself done no work on paralytic rabies in cattle, he well remembered the investigations carried out at his Institute, and the demonstration that the disease was actually rabies and that it was carried by bats.

He said that when he arrived last week on his first visit to London he hardly expected to be asked to speak at this meeting but he would like to describe a Brazilian way of catching bats—one goes out at night, with a long bamboo :

this is vibrated rapidly in the air and attracts the attention of the bats, which are killed by the rapid movement of the bamboo when they fly against it.

Professor J. C. G. Ledingham : There is, I am afraid, no time to take up the many intriguing virus problems which Dr. DE VERTEUIL's fascinating communication has raised and I must content myself with expressing my great appreciation of the important work which he and his colleagues have carried out in the attempt to control the vampire plague in Trinidad. By studying the activities of vampire bats in spreading rabies they have made notable contributions, I imagine, to our knowledge of the natural history and habits of these animals. I regret the absence of Dr. HURST, who might have discussed from first hand experience some of the new data relating to rabies infection in vampire hosts. I recall the evening four years ago when he produced the sections from the first monkey which succumbed to intracerebral inoculation with material from Trinidad, after exhibiting somewhat unusual and suspicious symptoms. As Dr. DE VERTEUIL has said, we were informed that the local diagnosis of the human cases was some kind of poliomyelitis or ascending myelitis, and of the cases in cattle, some kind of botulism. We were not, therefore, prepared for rabies. Typical Negri bodies were present and at once the question arose whether any one had been bitten by the monkey during its illness. One laboratory assistant had a few days before been bitten and he had to go through a course of antirabic immunisation, and as the investigation proceeded two or three others had to undergo this troublesome immunisation owing to some inadvertent pricking of the fingers through rubber gloves while performing autopsies on monkeys. The problem is one of vampire control and of the study of rabies in vampires, the latter now greatly facilitated by the fact that these animals can be kept in captivity and artificially fed. It would be of great interest to ascertain what constitutes the ultimate reservoir of the rabies virus, whether it is in the bat or possibly in carnivora, as I understand infection may be carried by bats flying over from Venezuela. We are also likely to obtain as the investigation proceeds some definite information on the very important question of virus carriers in nature—a question on which recent work on psittacosis is already throwing light. We shall look forward with great interest to further developments of these investigations in Trinidad.

Dr. W. H. Andrews : With respect to the distribution of the disease, I understand that some years ago Brazilian workers who were studying the condition near the frontier of British Guiana, also studied cases in cattle on the British side of the border, and found this peculiar form of rabies to exist there. It is of considerable interest from the veterinary side to note, however, that some time later an outbreak of cattle disease in the same area of British Guiana was investigated by the Government Veterinary Surgeon, who did not find this form of rabies, but a condition that appeared to be probably Aujeszky's disease, or infectious bulbar paralysis. There was a considerable clinical

difference, this form of Aujeszky's disease being characterised especially by intense pruritus. As far as domesticated animals are concerned, this form of rabies, or rabies-like disease, has become of enormous importance to South America, and it is interesting to note in the paper by MIGONE and PEÑA written in 1932, that a few sporadic cases had apparently been occurring in Paraguay for at least 20 years, but that it was only in the last 5 years that the disease had attained serious proportions.

A virus from Trinidad has been maintained for several years at the Weybridge laboratory, and a point of interest is that the virus has been found easier to maintain in guineapigs than in rabbits. The transmission in guineapigs has proved to be considerably more regular, and another point of interest and some importance is that we have not found it necessary to expend time and trouble on subdural inoculation. The virus has been kept going quite well by subcutaneous inoculation into areas, such as the cheek, which are rich in nerves. I would have been inclined to expect few, if any, cases of the disease in the cat, on account of its nocturnal habits, and only very few cases in the dog, because of that animal's custom of sleeping "with one eye open." One would not expect these two species to give the bats many opportunities, but quite apart from that aspect the dog appears to be very resistant to the virus, or, at any rate, to the strain which we have at Weybridge. Material which proved definitely infective to cattle and sheep has failed to infect the small number of dogs inoculated.

Dr. de Verteuil (in reply): I thank Dr. LINDSAY very much for the interesting report he has given us about Paraguay. There seems to be no doubt, from that report, that the disease there is definitely rabies. It certainly is most interesting to hear that there was a large epidemic of canine rabies there in 1926, and again in 1931. The remarks which I wish to make in this connection also refer to a point brought up by Dr. ANDREWS. In actual fact dogs are fairly commonly bitten by the bats in Trinidad; and although I agree with Dr. ANDREWS that decent dogs sleep "with one eye open," the type of dog we mostly have in Trinidad usually has both eyes closed, and many of them are the most sickly looking curs you could imagine. A fair number of dogs have been kept under observation, suspected to be suffering from symptoms suggestive of rabies, but in every instance the diagnosis of rabies has been eliminated. The point I should like therefore to make is this—is it possible for a rabid dog to bite a bat whilst it is lapping up its blood? Dr. ANDREWS thinks not, but I do not see why it should be regarded as impossible, especially if the particular dog is such a specimen as described above. If therefore we think it possible for a vampire bat to be infected in this way by a rabid dog it would be interesting to consider whether the dog virus after several passages in the bat could become so modified as to produce in man those symptoms of paralytic rabies which are so typical of this disease to-day: I see no reason why that point should not be tested experimentally.

With regard to the remarks of Dr. TORRES: I was interested in his description of his method of killing bats. A similar method seems to be practised in the East—I have seen it shown on the screen in cinemas—where they kill flying foxes by having two long poles with a net stretched across. A number of boys get near the trees where the flying foxes roost, and raise an alarm, with the result that the bats get scared, and fly off and bang their heads against the net. These bats are said to be palatable and are usually eaten. It is much the same idea, but I should like to try the method Dr. TORRES described. It would be useless, however, for the vampires, but it might do for the insectivorous and fruit-eating bats: of course, we do catch many bats with hand nets.

Professor LEDINGHAM's suggestion about a possible reservoir is very interesting. As a matter of fact we have always had in mind, that the fruit-eating bats might serve as reservoirs, and it was mainly on that account that we started the work in connection with the fruit-eating bats. We know of cases in which infected fruit-eating bats were caught fighting with each other, and presumably it would be easy for such bats to infect *Desmodus* as well as each other.

With regard to the possibility of carnivorous animals being infected in Trinidad, I do not think there is much chance of that, though it has been proved in Brazil that tapirs and other animals can be infected. We have kept a special watch on the mongoose, but so far we have never seen any infected ones. Of course, when the mongoose gets infected in the usual way, it is known to be a dangerous animal.

With regard to the possibility of *Desmodus* bats flying to Trinidad from Venezuela, my own view is that these bats do not fly ordinarily more than a mile or two, and that this is not likely; though, of course, there might be such a thing as a migration of bats. There is, however, every chance that they might come over in cattle boats from Venezuela or British Guiana, and we suspect strongly that that is the way by which the disease has been introduced into Trinidad.

The meeting was preceded at 7.45 by a Demonstration of specimens of bats, photographs, charts and maps, arranged by Dr. DE VERTEUIL.

The following contribution was received after the meeting from Dr. HURST of the Lister Institute.

Dr. E. Weston Hurst: As Dr. DE VERTEUIL has said there is no possible doubt that the Trinidad disease is rabies. Although in South America the term "mal de caderas" has apparently been used in connection with paralytic rabies, pseudorabies and a trypanosome infection, even the inexpert should

experience no difficulty in the differential diagnosis of these three wholly dissimilar maladies. HURST and PAWAN suggested previously that the Trinidad disease was possibly due to a rabic virus modified by passage through the tissues of the bat. One ground for this assumption was the relative difficulty in establishing the virus in the rabbit as contrasted with the monkey and the guineapig. I have since experienced the same difficulty with a rabic virus (from a case of paralytic canine rabies) isolated in New Jersey, U.S.A., so that this behaviour is not peculiar to the Trinidad virus. Again, as Dr. DE VERTEUIL suggests, the very frequent occurrence of the paralytic form of the disease in man may possibly be correlated with the long exposure of the wound on an extremity to the infective material, *viz.* the bat's saliva, and is not necessarily indicative of an unusual strain of virus. But the apparent difficulty in infecting the dog with the Trinidad virus is certainly remarkable.

The point most worthy of comment in Dr. DE VERTEUIL's paper, however, is the suggestion from the work done in Brazil that in the vampire bat rabies may assume a clinically latent form, while for months the saliva may be virulent and the animal transmit the disease. If this is finally proved to be the case it will be a discovery of the greatest importance. It is well known that the saliva of a rabid dog may be virulent for a day or two before clinical symptoms develop, but in no mammalian host has there hitherto been any indication that the nervous disease and death are long delayed after the salivary glands have been infected. REMLINGER and BAILLY* have shown that in a reptile (*Testudo mauritanica*) intracerebral inoculation is not followed by symptoms of rabies despite the fact that the virus may be recovered from the brain for as long as 302 days. It is also said that in the hibernating hedgehog the incubation period of the disease is greatly lengthened. But neither of these observations appears to have any real bearing on this most interesting problem, which will doubtless be adequately explored in the near future.

*REMLINGER, P., and BAILLY, J. (1932.) *Ann. Inst. Past.*, xlix, 665.

Proceedings of a Clinical and Laboratory Meeting held at the Hospital for Tropical Diseases, Endsleigh Gardens, Gordon Street, London, W.C.1, at 8.15 p.m., on Thursday, 12th December, 1935.
SIR ARTHUR BAGSHAW, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

DEMONSTRATIONS.

Major H. C. Brown.

Serological diagnosis of Weil's disease.

Major H. C. Brown gave a demonstration of the agglutination and adhesion tests used in the serological diagnosis of Weil's disease.

Charts were shown demonstrating the presence of agglutinins in the sera of patients during the first week of the disease, also the ascending titre of the serum as the disease progressed.

Dr. Mather Cordiner.

Demonstration of X-ray photographs illustrating various pathological conditions.

Radiograms to illustrate the following conditions were shown :—

1. Normal mucous membrane relief of the stomach.
2. The mucous membrane relief in cases of gastritis.
3. The various factors contributing to ulcer niche formation.
 - (a) Mucous membrane autoplastik.
 - (b) Oedema of mucous membrane.
 - (c) Cicatrical changes in gastric wall.
4. Early mucosal changes in gastric carcinoma.
5. Malignant degeneration of simple gastric ulcer.
6. Benign growths of stomach.

Dr. N. Hamilton Fairley.

(1) Jaundice in sewer workers.

Charts illustrating the temperature variations and fluctuating bilirubin and urea values in two cases of Weil's disease in London sewer workers were shown. In both instances leptospirae were isolated by blood culture, and the rise in the agglutinin titre of the serum was investigated by Major H. C. Brown.

In one patient a dramatic response followed convalescent serum given intravenously. The case histories are being published in detail elsewhere.

(2) Case of idiopathic steatorrhoea associated with megacolon.

Mr. W. W., a butler, aged 55 years, was admitted to the Hospital for Tropical Diseases on 21st June, 1935, with a history of morning diarrhoea, flatulent distension, cramps in the hands, soreness of the tongue and great loss of weight. Until 18 months ago he was perfectly fit and had never been to the tropics. There was anaemia, great emaciation and marked thinning of the abdominal wall with distension and visible peristalsis. Blood pressure—S/D = 100/80. The tongue showed atrophy of the filiform papillae in patches.

Laboratory Examination.—Laboratory investigations showed hyperchlorhydria, a megalocytic type of Price-Jones curve, flat glucose tolerance curves, steatorrhoea and hypocalcaemia (8.2 mg. per 100 c.c.). Total fat = 43.7, of which 85.7 per cent. was split. R.B.C.'s = 2,780,000 per c.mm.: haemoglobin = 64 per cent.: colour index = 1.1. Two glucose tolerance tests taken at different times were of the flat type in contradistinction to the intravenous glucose curve which rose higher and showed only a sluggish return to its original level when compared with the control.

These findings do not agree with those of THAYSEN* and indicate that glucose was being slowly utilised by the tissues; it favours the view that the flat glucose curves, observed after 50 grammes of glucose *per os*, result from malabsorption of this monosaccharide. The sluggish utilisation is dependent on malabsorption which has the same effect as carbohydrate deprivation.

X-ray Examination.—X-ray examination showed marked megacolon, but no evidence of osteoporosis in the long bones or pelvis. There was coarsening of the mucosal pattern in the small intestine.

Treatment.—Under intensive liver extract therapy the anaemia was completely recovered from, but although blood counts were normal the patient died with severe diarrhoea and tetany (calcium = 5.8 mg. per 100 c.c.: phosphorus = 2.5 mg. per 100 c.c.) despite all treatment. Such a result is in his (Dr. FAIRLEY's) experience not seen in tropical sprue.

Interesting features were: (1) The absence of a history of alimentary trouble in childhood. (2) The hyperchlorhydria. (3) The associated megacolon. (4) The flat glucose curves due to malabsorption. (5) The adequate haematological response despite the persistent diarrhoea and tetany. (6) Fatal issue at a time when the R.B.C.'s = 4,960,000 per c.mm. and the haemoglobin = 100 per cent. (HALDANE).

(3) Bilharzia complement fixation reaction—persistence of circulating antibody after treatment.

Case 1. This patient gave a history of bathing in the same pool as her brother who contracted rectal schistosomiasis. The complement fixation reaction was strongly positive (25 M.H.D.'s) and there was an eosinophilia of 6.5 per cent.

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Ova were never found in the excreta, intestinal symptoms were not present, and not improbably the case is an example of exclusive infection with male schistosomes. Reaction still positive 4 years later.

Case 2. Case of chronic vesical schistomiasis with haemospermia and involvement of the bladder, prostate, seminal vesicles and right ureter which was constricted and dilated. Pain in the right loin persisted and the reaction was still positive (5 M.H.D.'s) some 12 months later.

Case 3. Naval officer with *Schistosoma japonicum* infection contracted while snipe shooting on the Yangste. In all, four courses of treatment were given, the reaction finally becoming completely negative in 42 months.

Case 4. Naval officer with *Schistosoma japonicum* infection contracted while shooting on the Yangste. He suffered from bilharzial granuloma of the brain, in which ova were found by Professor LEIPER. The reaction finally became negative 54 months after a full course of tartar emetic treatment intravenously.

Four other cases (two *S. haematobium* and two double infections with *S. mansoni* and *S. haematobium*) gave serological reactions which remained positive throughout the period of investigation. One case was followed for as long as 46 months, and even after this long interval 10 M.H.D.'s of complement were fixed by the patient's serum in the presence of *S. spindale* antigen. Ova were not found again in the faeces or urine of any of these cases.

Experimental investigations in goats infected with *S. spindale* revealed a survival of male schistosomes in certain of these animals after all females had been destroyed by specific anthelmintics, and one possible explanation of these persisting positive reactions is survival of male schistosomes within the portal system of man.

In some bacterial infections, however, such as typhoid and undulant fever, complement-fixing antibody may persist for years after eradication of the infection and it is possible that the explanation lies along similar lines.

Dr. N. Hamilton Fairley and Mr. R. J. Bromfield.

Blood sugar curves in tropical sprue.

Case 1. R.W., chronic sprue with bulky, loose, fatty stools, sore tongue, megalocytic anaemia and typical sprue abdomen.

Total faecal fat = 48.6 per cent., of which 77.6 per cent. is split. R.B.C.'s = 2,590,000 per c.mm. : haemoglobin = 62 per cent. : colour index = 1.2 : leucocytes = 3,800 per c.mm.

The only exceptional feature is that hyperchlorhydria is present (80 N/10 HCl).

Case 2. K.M., aged 47 years. Onset in Ceylon in 1927. Now complains of diarrhoea and of sore tongue and mouth. Has lost 28 lbs. in weight, is anaemic and the stools are loose and frothy. Abdomen typical of sprue.

R.B.C.'s = 2,300,000 per c.mm.: haemoglobin = 50 per cent.; colour index = 1.1: average diameter of corpuscle = 8.4μ : leucocytes = 4,000 per c.mm.; blood picture typical of megalocytic anaemia.

- (i) Total fat = 41.2 per cent., of which 62.8 per cent. is split.
- (ii) Achlorhydria with response to histamine.
- (iii) Van den Bergh = indirect + (4.5 units).
- (iv) Blood calcium = 8.8 mg. per 100 c.c.

Charts were demonstrated showing the flat glucose tolerance curve in both these cases and the improved curve after treatment. The intravenous curve rises higher and takes longer to return to its original level than the normal curve in each instance.

In contra-distinction to THAYSEN's findings these results support the view that sugar is not being excessively rapidly utilised by the tissues, and that malabsorption is the basis of the flat glucose tolerance curves in this disease.

Dr. G. W. M. Findlay.

Sections illustrating chorio-lympho-meningitis, a new virus disease of mouse and man.

This disease was first described in the United States of America by ARMSTRONG in 1934. It is due to a filterable virus which infects mice, but does not produce symptoms unless it gains access to the brain when it causes infiltration of the meninges and choroid cells with lymphocytes. When inoculated intracerebrally mice die from 6 to 8 days later. The virus is excreted by the urine and is found in the blood, kidneys, liver and spleen. The virus instilled intranasally in mice causes death: it can pass through the lightly scarified skin of the mouse. Guinea-pigs and rats are susceptible and also man. Human cases have been described in the United States and the virus has now been isolated from two patients in London. The symptoms are those of an influenza-like disease with fever followed by weakness in the legs, neuritis and evidence of meningitis. The patients recover. Monkeys are extremely susceptible and almost invariably die. There may be infiltration in the suprarenals.

Dr. G. Carmichael Low.

Clinical cases.

1. A case of locomotor ataxia in a Japanese.
2. A case of Weil's disease.
3. A case of laryngeal obstruction with an accompanying helminthic infection.

Dr. F. P. Mackie.

(1) Pathological specimens illustrating leptospirosis in man and animals.

Sections of human and guinea-pig liver and spleen were shown in which marked necrotic changes were evident. *Leptospira* were abundant in the

guineapig liver, scarce in the kidney, but could not be demonstrated in the human organs.

(2) Macroscopic and microscopic preparations from Dr. Fairley's case of idiopathic steatorrhoea.

Macroscopic specimens showed the small size of the heart ($4\frac{1}{2}$ oz.) a very small spleen ($1\frac{1}{4}$ oz.) and the upper part of the femur in which the marrow was hyperplastic as in pernicious anaemia. The intestinal tract showed no microscopic or naked eye change at any level.

(3) Sections from fatal case of sickle cell anaemia.

The organs were from a Portuguese child, who died of profound anaemia, and had been sent for diagnosis by Dr. STEVEN of Demerara. An extreme form of sickling of the red cells was present in the spleen sinuses but very little was seen in other organs.

(4) Macroscopic and microscopic preparations from a case showing (a) primary carcinoma of the lung; (b) amyloid degeneration of the spleen and kidneys; (c) ? regional ileitis ("Crohn's disease").

These specimens were from a man who was thought to have malignant disease of the intestinal tract on account of the passage of blood in the stools. At the autopsy he was found to have a primary carcinoma of the lung and well marked amyloid discase of the spleen and to a less extent of other organs. His intestinal symptoms were due to the presence of an area of inflammatory hypertrophy with destruction of the mucosa involving about a foot of the jejunum which was not due either to tuberculosis or malignant disease. This lesion suggests a condition of regional ileitis (Crohn's disease).

(5) Heart of a child embedded in mediastinal new growth—? lymphosarcoma of thymus.

This specimen was presented by Dr. G. H. STEVEN, of Georgetown, Demerara.

Dr. P. Manson-Bahr.

(1) A case of leprosy showing fibrotic nodules on the lower limbs.

The patient had contracted the infection in India about 1926: he returned to England in 1932, but was not diagnosed as a subject of this disease till May, 1935, when he attended the Hospital for Tropical Diseases. The case is one of mixed anaesthetic, nodular and macular forms. Several hard fibrotic nodules were present on the wrists and legs. On biopsy they were found to contain the typical cell picture with masses of acid-fast bacilli. The improvement on systematic treatment has been remarkable. Treatment consisted of three protein shocks plus a total of 41 c.c. ethyl chaulmoograte injected deep subcutaneously.

(2) A case of blackwater fever with persistent high blood urea. Recovery after multiple blood transfusions.

This case was admitted moribund with incontinence of urine and faeces and with loss of corneal reflexes. The haemoglobin was less than 20 per cent.

and the red blood cells 900,000. Recovery took place after intravenous injections of sodium bicarbonate, 20 per cent. glucose and three blood transfusions. The blood urea steadily rose to a maximum of 480 mg. per cent. on the 11th day. Showers of hyaline casts from the urinary collecting tubules appeared in the urine and immediately the percentage of urea dropped. No signs or symptoms of uraemia were noted.

(3) Photograph illustrating lymphatic obstruction in a case of *Loa loa* (*Filaria loa*) infection.

This patient had served on the Gold Coast for 11 years, prior to which he had spent 5 years in Palestine. He had an eosinophilia of 50 per cent. and scanty *Microfilaria loa* were found in his blood. Night blood was negative. There was a definite lymphatic swelling of the right leg with crysiparetoid condition of the skin which was hot and tender with solid oedema. There was a left hydrocele the size of a pear. The right leg was 2 inches bigger in circumference than the left from the knee downwards. There were no enlarged glands. The swelling is now rapidly subsiding leaving no trace of lymphatic obstruction as in *Filaria bancrofti* infections.

(4) A case of anaemia following sprue.

The patient had sprue in 1931, made a complete recovery in 1932. No anaemia was noted till 1935, when it rapidly developed without loss of weight and without return of sprue symptoms. Fractional test meal: marked hyperchlorhydria. Blood examination showed megalocytic anaemia with Cabot's rings. These rings are believed to be the remains of the nuclear membrane, the cells containing them show evidence of immaturity and contain stippled material. This is probably a case of achrestic anaemia such as that recently described by WILKINSON which, as did this case, responded immediately to parenteral injections of liver extract, while there was, as in this case, no response to liver therapy when given by the mouth.

Professor J. G. Thomson.

(1) Large macrophages simulating *Entamoeba histolytica* in the lymph follicles of the human appendix.

In an editorial of the *East African Medical Journal* of February, 1935, it was stated that Dr. VINT and Dr. WILKINSON had demonstrated that the appendix is frequently the seat of chronic amoebic infection. This was followed however, by a letter by Dr. VINT (1935) pointing out that the bodies within the lymph nodes, originally supposed to have been amoebae, were simply macrophages and that no evidence of amoebic infection could be demonstrated in a large number of appendices removed by operation.

WILKINSON (1935) next suggested that although the amoebae might not actually be present in the appendix nevertheless an associated amoebiasis of the colon might predispose to an attack of appendicitis. Such a conclusion

is apt to be misleading for there is nothing to suggest that there is any relationship between appendicitis and amoebiasis. On the other hand it is quite a possibility that appendicitis might be mistaken for amoebiasis of the caecum or that a history of amoebiasis might temporarily lead to confusion with acute appendicitis. Actual infection of the appendix with *Entamoeba histolytica* seems to be a relatively rare occurrence and has been noted by MUSGRAVE (1910), HOGAN (1920) and GREAVES (1933).

The specimens shown under the microscopes were sections through the appendices removed at operation, which had been kindly sent by Dr. VINT from Nairobi. They showed a hyperplasia of the central part of the lymph nodes containing numerous large mononuclear cells (macrophages) with many cytoplasmic inclusions. Such mononuclear cells, which must not be mistaken for amoebae, are common in the lymph nodes of the intestine of typhoid fever and other conditions associated with inflammation. Similar mononuclear cells with inclusions may occur in the lymph nodes of the spleen of monkeys which have succumbed to malarial haemoglobinuria. The origin of these large mononuclear cells is a matter of doubt but the general opinion is that they are derived from the reticulo-endothelium. Evidently these cells have frequently been mistaken for pathogenic amoebae especially in sections, but in their nuclear structure and general morphology they really bear no resemblance to *Entamoeba histolytica* except that they are actively phagocytic especially for lymphocytic cells.

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(2) Phagocytosis of the schizonts of *Plasmodium falciparum* by polymorphonuclear cells.

The phagocytosis of schizonts of *P. falciparum* has been frequently seen in the peripheral blood stream especially in massive infections accompanied by coma of the patient and it has been suggested that the polymorphonuclear cells, and also the monocytes are engaged in removing dead parasites. It seems to be perfectly clear, however, that the polymorphonuclear cells have a special affinity for fully developed live schizonts of *P. falciparum* and that they ingest these with great avidity. One polymorph will account for as many as eight schizonts. The placental smears demonstrated under the microscopes showed the remarkable capacity of these polymorphs for engulfing schizonts and, therefore, it would seem that this cell can play on occasion a very important part in the control of an infection.

Dr. Lucy Wills.

The occurrence of bodies of doubtful nature in the red blood corpuscles of splenectomized monkeys.

Two types of "bodies" occurred in the red blood corpuscles of monkeys (*Macacus rhesus*) following splenectomy. The first were round dots about the size of a Howell-Jolly body, which stained dark purple with Giemsa and resembled *Anaplasma marginale*; they varied in size and occurred singly or in twos or threes and sometimes showed budding. This type was first seen 8 to 12 days after operation. Later the second type appeared and became increasingly frequent week by week; this type consisted of small, multiple forms which might be rod or comma-shaped or in rosette or ring form, all of which stained blue with Giemsa. Some of these forms resembled *Bartonella* bodies. The "sphere" forms were supravitaly stained with neutral red; the multiple forms did not appear to take up this stain. Till the bodies can be transmitted to other animals or cultured their nature must remain in doubt, but their appearance was not related to a severe anaemia (in which condition nuclear fragments or Howell-Jolly bodies are frequently observed) but was a regular appearance after splenectomy in the four animals operated on. The "sphere" forms were observed once only in two intact animals, but the number of red cells affected was very few: these monkeys were suffering from a mild degree of anaemia.

COMMUNICATIONS.

HUMAN BRUCELLOSIS IN TANGANYIKA TERRITORY.

BY

D. E. WILSON,*

Medical Officer, Tanganyika Territory.

The above title has been chosen with a view to dispersing the confusion which attaches to the nomenclature of diseases caused in man by *Brucella* organisms. The words "undulant," "Malta" or "Mediterranean" fever may no longer properly be applied to a condition which is world-wide in its distribution and capricious in its symptomatology. When there is added to this the fact that the so-called "abortus fever" is also included amongst the manifestations of *Brucella* infections, it may be submitted that the time is ripe for the inclusion of all such infections in one comprehensive category. For this purpose the all embracing description "brucellosis" made use of by GILTNER (1934) is suggested.

In a previous paper WILSON (1935) showed that *Brucella* infections were distinctly prevalent in a cattle-bearing district of Tanganyika Territory. The district referred to was the Mpwapwa district of the Central Province. Continuing his investigations a total of 317 sera were tested. Of these 23 showed the presence of *Brucella* agglutinins in titres varying from 1:25 to 1:20,000. Since publishing his paper sera have been found positive in other districts of the Territory namely Tanga, Tukuyu and Kilosa.

THE TYPE OF INFECTION.

Of six blood cultures undertaken, four were positive and satisfactory subcultures were obtained from three of these. I found the most satisfactory method of blood culture to be direct inoculation on to liver agar. A large culture flask—Roux's pattern—was inoculated with 8 to 10 c.c. of the patient's blood and the blood spread over the entire surface of the medium which represented an area of 220 sq. cm. In all the positive cases growth was apparent after 5 days. No culture was discarded until it had been incubated for 28 days. The cultures were made aerobically with no increased CO₂ tension.

The subcultures obtained were numbered as follows: T.H.I., 256, and 260, and sent to the Veterinary Research Laboratory, Kenya Colony, where they were typed by courtesy of the Chief Veterinary Research Officer. The results were very interesting showing that we have in Tanganyika both *melitensis* and

*I have to thank the Honourable the Director of Medical Services, Tanganyika Territory, for permission to publish this paper.

abortus types of human brucellosis. JEWELL (1931) has reported a case of "undulant fever" in Kenya due to *Br. abortus* but I can find no record in East Africa of a case in which *Br. melitensis* was the causative organism.

The three cultures referred to above reacted as follows:—

			T.H.I. as a typical <i>Br. abortus</i> .
256	"	"	<i>Br. melitensis</i> .
260	"	"	<i>Br. melitensis</i> .

It will be noted that no increased CO₂ tension was necessary for the growth of the human strain of *Br. abortus*. This is comparable with the findings recorded by DUNCAN (1928) and BEVAN (1930) in the case of certain Rhodesian strains.

In both *melitensis* cases one could get no history of the patients having drunk goats' milk and it is against the local custom of the Mpwapwa natives to do so. How did they contract the infection? Are the Mpwapwa goats infected with *melitensis* or is the local bovine strain a *melitensis* type? The Veterinary Pathologist, Tanganyika, is at present investigating the subject and he has already found the sera of one or two slaughter goats to contain *Brucella* agglutinins. In Malta, RAINSFORD (1932) has suggested dust as a possible source of infection. Mpwapwa district is one of the driest districts of Tanganyika and is wind-swept and very dusty. Dust containing infected goat's urine and faeces may thus be the source of the *melitensis* infection.

CLINICAL SYMPTOMS.

The clinical symptoms were similar to those recorded in text books and various papers and will be described briefly.

Intermittent fever reaching as high as 104·8° F. with daily fluctuations of as much as 8 degrees. It is interesting to note that a patient may have a temperature as high as 104·2° F. and yet deny that he *felt* feverish. HARDY (1929) found similar results in some of his cases. The pulse rate was slow in proportion to the temperature. Great muscular weakness with cramps in the calves and loss of flesh resembling in the later stages advanced tubercular emaciation. Frontal headache with dizziness and dimness of vision was a distressing complaint. The headache increased in severity as the temperature reached its fastigium. A very characteristic symptom common to all the cases was a feeling of numbness and burning pains in certain joints. The joints chiefly affected were the wrist, elbow and ankle. Fleeting, sharp, neuralgic pains in the limbs were also complained of.

Chronic constipation was always present: in one case enemata had to be given for 10 weeks. Anorexia was not apparent in any of my cases.

Involuntary twitching of muscles and fibrillary tremblings of the skin were observed.

In my *melitensis* cases there was a characteristic sour, moist odour with a powdery desquamation of the skin.

There was slight enlargement of the spleen in three cases but in no instance was the liver enlarged.

The blood picture presented the usual features associated with human brucellosis, namely a leucopenia with a relative lymphocytosis.

	Total white blood corpuscles.	Lymphocytes.
No. 256	2,500	85 per cent.
„ 260	3,750	51 „
„ 272 (type unknown)	3,250	80 „

In the one known *abortus* case, T.H.I., the above symptoms were present but the severity was greatly reduced and a lymphocytosis of only 41 per cent. was recorded.

TREATMENT.

The treatment of human brucellosis still appears to be a question requiring considerable research. The bacteriology, serology, haematology, epidemiology and symptomatology have occupied the time of large numbers of research workers in many countries but there appears to have been very little advance in treatment. Although the disease in its most virulent form has a low mortality rate of 3 to 4 per cent. it is a most painful, wearying and debilitating disease and one in which the physician has to stand aside and hope that to-morrow or the next day will see a change for the better in his patient. The disease may also pave the way for the development of tuberculosis. It is interesting to note that BROCK (1930) has observed several cases of human brucellosis in which there were signs and symptoms pointing to pulmonary tuberculosis—the diagnosis of tuberculosis being confirmed at a later date.

CONTERNO (1930), LE CHUITON and NÉGRÉ (1930), THURBER (1930), BETHOUX (1930), DARRÉ and LAFFAILLE (1928) and HOFFMAN (1929) appear to have had a certain amount of success with the intravenous use of trypaflavine and other acridine salts.

The present writer found trypaflavine to be of no value. RAINSFORD (1935) describes his results with trypaflavine as very disappointing.

CASE T.H.I.

This patient received a total of 1.5 grammes of trypaflavine given in a 2 per cent. solution intravenously as follows :—

26.2.35	5 c.cm.	15.3.35	10 c.cm.
2.3.35	5 „	19.3.35	10 „
6.3.35	5 „	22.3.35	10 „
9.3.35	10 „	26.3.35	10 „
12.3.35	10 „		

At the end of the treatment the patient had not improved. Three other cases failed to respond to this treatment.

The following drugs were also tried but without success :—(1) Sulpharsphenamine intramuscularly. (2) Bayer 205 intravenously. (3) Novarsenobillon intravenously.

MANSON-BAHR (1935) has reported favourably on the intravenous use of T.A.B. vaccine as a means of increasing the euglobulin content of the serum and thus promoting phagocytosis of the offending *Brucella*.

This treatment was tried out in four of my cases (272, 256, 302 and 559). In Case 272 the result was good, the patient's temperature remaining normal for three weeks—a further history was not obtainable.

In Case 559 (which was a laboratory infection in a European diagnosed by blood culture as a *Br. abortus* infection) the result was also good.

In the other two cases the results were no improvement. Clinically from the severity of their symptoms three of the four cases appeared to be of the *melitensis* type.

CASE 272.

15.3.35, diagnosed—(had been acutely ill for only two weeks).

18.3.35 received 5 c.c. of his own blood intramuscularly—no improvement on 26.3.35.

27.3.35 received 2,000 million T.A.B. intramuscularly.

1.4.35 received 1,000 million T.A.B. intravenously.

After the intravenous injection the patient had a severe rigor and sharp rise of temperature which fell to normal in 24 hours and as stated above did not rise again. His general condition improved rapidly and when he was discharged from hospital he appeared to be quite normal. His agglutination titres on admission were :—

Br. melitensis 1 : 2,500

Br. abortus 1 : 1,000

On discharge his titre had fallen to 1 : 125 for *Br. melitensis*. It is also interesting to note that his total white blood corpuscles rose from 3,250 per c.mm. to 13,000 after the intravenous injection.

CASE 559.

This patient had been acutely ill for about 4 weeks before receiving protein shock.

On 12.6.35 he received 50 million organisms (T.A.B.) intravenously and had a sharp rise of temperature but no rigor.

On 15.6.35 a further dose of 100 million was injected intravenously. On this occasion there was a slight rigor and the temperature rose to 103.8° F. The leucocyte count before and after this injection is interesting.

15.6.35 Before injection 5,000

Some hours after injection 6,250

16.6.35 7,600

After the reaction due to the second injection had subsided the temperature fell to normal and the patient improved rapidly and the date of writing, 28.8.35, is perfectly fit.

CASE 256.

This patient, who had been actually ill for 6 months previously, received the following treatment :—

19.3.35	Autogenous vaccine of <i>Br. melitensis</i>	0.1 c.cm.
22.3.35	" "	0.2 "
25.3.35	" "	0.5 "
29.3.35	" "	0.8 "
1.4.35	" "	1.0 "
4.4.35	" "	1.0 "
8.4.35	" "	1.0 "
12.4.35	" "	1.0 "
22.4.35	" "	1.0 "

(0.1 c.c. contained 150 million organisms.)

This patient was also given an intravenous injection of 1,000 million T.A.B. on 15.4.35; he had a sharp reaction but did not improve, and there was no marked leucocytosis. The treatment by an autogenous vaccine also proved useless.

CASE 302.

This was a laboratory infection and the severity of the symptoms pointed to a *melitensis* infection. The patient received six injections of T.A.B. intravenously commencing with a small dose of 50 million and gradually increasing the dose to 1,200 million. The patient was diagnosed as a case of undulant fever by agglutination when the titre of his serum was :—

Br. melitensis 1 : 5,000

Br. abortus 1 : 5,000

He had previously been ill for a period of about 10 days. His treatment was as follows :—

	Million T.A.B. intravenously.
22.6.35	50
24.6.35	100
27.6.35	200
30.6.35	300
3.7.35	400
22.7.35	1,200

He had a rigor and rise of temperature with a slight leucocytosis on all occasions. After the last injection his temperature rose to 106° F. but there was no delirium or other alarming symptom. In spite of all these protein shocks the patient remained acutely ill and to date, 18.8.35, his temperature has remained high and his serum is now agglutinating both *Br. melitensis* and *Br. abortus* in a titre of 1 : 20,000.

DISCUSSION AND SUMMARY.

1. Both the *Brucella melitensis* and *Br. abortus* type of undulant fever exist in Tanganyika Territory.

2. A Tanganyika human strain of *Br. abortus* required no increase of CO₂ tension for primary culture.

3. A suggestion has been put forward that the *melitensis* infections may have been disseminated by dust containing the dried urine and faeces of goats.

4. Various forms of treatment have been discussed: most gave negative results. Trypaflavine, Bayer 205 and novarsenobillon intravenously have proved useless as also has sulpharsphenamine intramuscularly.

Following "shock" treatment with T.A.B., two patients manifested definite clinical improvement, followed by rapid recovery, but of course it is not possible to assert that this improvement was the result of the particular treatment employed. I think, however, that this treatment is worthy of trial in early cases.

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THE PATHOGENICITY OF *ENDAMOEBEA HISTOLYTICA*.

BY

HENRY E. MELENEY

AND

WILLIAM W. FRYE.*

The pathological effects of *Endamoeba histolytica* differ widely both in different individual hosts and in different endemic localities. It is a well-known fact that even in the presence of a severe epidemic of amoebic dysentery such as occurred in Chicago in 1933 (BUNDESEN, TONNEY and RAWLINGS, 1934) many of the persons who become infected develop no symptoms referable to the amoeba. Others develop mild symptoms and still others develop severe lesions leading to acute dysentery and its complications. This difference in the response of individual hosts to the same strain of amoeba was demonstrated experimentally in human subjects by WALKER and SELLARDS (1913) in the Philippines and has been the experience of many other workers in the course of animal experiments.

The exact cause of this variation in the response of individual hosts has not yet been determined. It is probably the result of a number of factors which influence the resistance of the tissues to invasion. The general condition of the host, the hydrogen ion concentration of the intestinal contents, the bacterial flora, the diet, the effect of alcohol, the occurrence of other pathological lesions in the intestine, the presence of specific or non-specific protective substances in the blood—all or any of these factors may operate in determining the entrance of the amoebae into the tissues or in encouraging or inhibiting their destructive action on the tissues after entrance.

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The question as to whether *E. histolytica* can multiply indefinitely in the lumen of the intestine without invasion of the tissues is still unsettled, although many observations have been made to throw light upon it. REICHENOW (1926) observed the course of an infection in himself in which *E. histolytica* and *E. coli* were both present. The absence of symptoms and the similar response of the two species to purges and to variations in the faecal specimens led him to conclude that in his case the two species were multiplying similarly and that there was probably no tissue invasion by *E. histolytica*. ANDREWS and ATCHLEY (1932) secured negative tests for occult blood in symptomless carriers of *E. histolytica* and gave this as evidence against tissue invasion. Although there are many reports of spontaneous amoebic dysentery in monkeys, DOBELL (1931) found no tissue invasion in a group of monkeys naturally infected with *E. histolytica* and we (unpublished observations) have had a similar experience except in one monkey dying of tuberculosis. HEGNER (1935) found no tissue invasion in four monkeys experimentally infected with a strain of *E. histolytica* from a case of amoebic dysentery originating in the Chicago epidemic. KESSEL (1928) found no lesions in pigs naturally infected with *E. histolytica*. If this amoeba can live as a commensal in the lumen of the intestine in animals, it is reasonable to suppose that it may sometimes do so in man as well.

On the other hand there is considerable evidence that in man tissue invasion may always be present if *E. histolytica* becomes established, even if no symptoms develop and if no macroscopic lesions are found at autopsy. We know of no report of a human case in which amoebae were found in the stools during life without the presence of tissue invasion at autopsy. MELENEY and FRYE (1934) reported a human autopsy in which no macroscopic lesions in the colon were found but in which routine tissue sections showed slight damage to the mucosa and invasion of amoebae into the submucosa. Furthermore, the fact that practically all persons harbouring *E. histolytica* without symptoms give a positive blood complement fixation reaction indicates that antigen has been absorbed by the tissues. This must be interpreted either as tissue invasion by the amoebae or as the absorption of unaltered amoeba antigen (toxin) from the lumen of the colon. Since it is known that certain antigens can be absorbed from the intestine without obvious injury to the mucosa and can give rise to antibody production (LEDINGHAM and SCHÜTZ, 1931), a decision as to whether or not *E. histolytica* can produce a positive complement-fixation reaction without invading the tissues must await further observations.

STRAINS OF *E. histolytica* DIFFERING IN PATHOGENICITY.

The literature contains a number of studies in which strains of *E. histolytica* were compared with regard to their pathogenic activity in experimental animals. BAETJER and SELLARDS (1914) stated that strains which were more mildly pathogenic for kittens came from patients having milder symptoms. SHIMURA (1918) obtained a lower incidence of infection in kittens injected with small races of

E. histolytica from symptomless carriers than with large races from acute cases of amoebic dysentery. WAGENER and THOMSON (1924) were able to infect kittens with only one out of nine strains of *E. histolytica* from "chronic carriers" in California but readily infected kittens with a strain from a chronic case of dysentery originating in the Philippines. DOBELL (1931) found that strains of *E. histolytica* from macacus monkeys either failed to infect kittens or produced mild or chronic infections from which the animals usually recovered. BRUMPT (1928) and SIMIC (1931, 1931a) adopted the name *E. dispar* for strains of amoebae morphologically identical with *E. histolytica* which they stated did not produce dysentery in man and produced only mild, superficial lesions in kittens. BRUMPT holds the opinion that these amoebae are accountable for the high incidence of *E. histolytica* in many parts of the world where amoebic dysentery is rarely found.

We have carried on an intensive study in Tennessee dealing with strains of *E. histolytica* obtained from various sources. In order to overcome the difficulty presented by differences in the response of individual hosts to the infection we have used twenty or more kittens in each series of experiments with each strain, and have standardized the material and technique of inoculation (MELENEY and FRYE, 1932, 1933, 1935). Each strain of amoeba has been isolated in culture, young transplants have been used, cultures have been inoculated directly into the ileum after laparotomy incision, and the size (200 to 600 grammes), diet and housing of the kittens have been uniform. The percentage of successful infections and the extent and intensity of the lesions produced have been used as criteria for measuring the pathogenic activity of each strain. The percentage of infections produced in different series of experiments with the same strain has varied rather widely, but the average severity of the lesions produced has been so nearly uniform that we believe it can be taken as a true index of the pathogenic power of the various strains. This index has been reduced to a numerical figure by the following method: the colons of successfully infected kittens have been classified according to the size, depth and extent of the lesions, Class 1 representing no macroscopic lesions although amoebae were present in the lumen of the colon, Class 9 representing extensive involvement of the entire colon with deep and shallow ulcers, and the intermediate classes representing the stages of severity between these two conditions. Since the duration of the infection influences the degree of development of the lesions we have introduced this factor into the calculation of the pathogenic activity of the strain of amoeba concerned. The number representing the "class" of the colon is divided by the number of days the kitten survived after inoculation, and the quotient represents the "degree of pathology produced per day." Thus in a kitten which lived 6 days after inoculation and presented a Class 8 colon at autopsy, the pathology produced per day would be $8/6 = 1.33$. The average of these figures for a series of kittens provides a "pathogenic index" which our experience shows is reliable for any one strain of amoeba. Table I shows the pathogenic indices obtained in experiments with four strains of

TABLE I.
PATHOGENIC INDEX IN KITTENS OF FOUR STRAINS OF *E. histolytica* MAINTAINED IN CULTURE
FOR THREE YEARS.

Average Number of Days since Isolation of Culture.	Number of Kittens Inoculated.	Kittens Developing Infection.		Pathogenic Index.
		Number.	Per cent.	
Strain A-1 Hills.				
220	38	12	31.6	0.58
600	20	4	20.0	0.54
957	21	8	38.0	0.57
1,023	24	7	29.2	0.53
1,053	20	6	30.0	0.58
Total	123	37	30.1	0.57
Strain A-2 Hills.				
222	19	15	79.0	0.49
600	20	5	25.0	0.60
958	22	7	31.8	0.65
1,082	21	8	38.1	0.58
Total	82	35	42.7	0.58
Strain B-1 Bottom-land.				
180	20	12	60.0	1.06
602	20	11	55.0	1.01
875	25	16	64.0	1.09
1,036	24	20	83.3	1.25
1,047	20	13	65.0	1.28
Total	104	72	69.2	1.13
Strain B-2 Bottom-land.				
186	20	13	65.0	1.15
612	11	5	45.0	1.35
1,071	20	12	60.0	1.31
Total	51	30	58.7	1.23

TABLE II.
PATHOGENIC INDEX IN KITTENS OF CULTURES OF VARIOUS STRAINS OF *E. histolytica*.

Designation of Strain.	Source of Strain.	Average number of Days since Isolation of Culture.	Number of Kittens Inoculated.	Kittens Developing Infection.		Pathogenic Index.
				Number.	Per cent.	
A-1	"Healthy carrier," hill country, Tennessee	220-1,053	123	37	30.1	0.57
A-2	Chronic intestinal amoebiasis (?), hill country, Tennessee	222-1,082	82	35	42.7	0.58
B-1	Acute dysentery, bottom-land, Tennessee	180-1,047	104	72	69.2	1.13
B-2	Acute dysentery, bottom-land, Tennessee	186-1,071	51	30	58.7	1.23
B-3	"Healthy carrier," bottom-land, Tennessee	85	20	9	45.0	0.78
Brent	"Healthy carrier," Nashville	112	32	4	12.5	0.50
Wright	Amoebic ulcer of skin, Nashville	170	21	12	57.1	0.89
C-1	"Healthy carrier," Chicago factory	120	24	17	70.9	1.54
C-2	"Healthy carrier," Chicago hotel	134	21	15	71.3	1.60
C-3	Acute amoebic dysentery, Chicago hotel	83	22	17	77.3	1.48
C-4	Acute amoebic dysentery, Chicago steel worker	107	22	19	86.3	1.70
N.R.S.	Dobell. <i>Macacus nemestrinus</i> — <i>M. rhesus</i> — <i>M. sinicus</i>	2,177	25	5	20.0	0.36
D.M.R.	Dobell. Acute dysentery (human)— <i>Macacus sinicus</i> — <i>M. rhesus</i>	2,174	20	6	30.0	0.92

E. histolytica over a period of 3 years in cultivation. It is evident from this table that there has been a fair degree of uniformity in the behaviour of each of these strains throughout the period of observation.

Using the same method we have determined the pathogenic index of nine other strains of *E. histolytica* from various sources, using a single series of twenty or more kittens for each strain. Table II shows the average results with the four strains mentioned above as well as the results with the other nine strains. Two of the first four strains, A-1 and A-2, came from a symptomless carrier and a person with mild intestinal symptoms in an isolated white rural community in the hill country of Tennessee where, in spite of a known incidence of *E. histolytica* of 38 per cent., there was no evidence of acute amoebic dysentery. It will be seen that these strains gave a relatively low pathogenic index in kittens. The other two of the first four strains, B-1 and B-2, came from acute cases of amoebic dysentery in a rural community in the bottom-land of Tennessee, containing both whites and negroes, where about twenty-five cases of acute amoebic dysentery had occurred recently, although only 19 per cent. of the people were found to harbour *E. histolytica*. These strains produced in kittens a pathogenic index about twice as high as the hill strains.

The next strain, B-3, came from a symptomless carrier in the same bottom-land community, and produced a pathogenic index about half-way between the hill strains and the other bottom-land strains. The "Brent" strain was obtained from a symptomless carrier in Nashville and produced a pathogenic index about equal to the hill strains. The "Wright" strain was obtained from an amoebic skin ulcer following a peritoneal abscess of undetermined origin, and produced a pathogenic index more nearly equal to the bottom-land strains. The next four strains, C-1, C-2, C-3, and C-4, were obtained from Chicago through the courtesy of the Health Department of that city. Two were from acute cases of amoebic dysentery and two from symptomless carriers. One acute case and one carrier had apparently become infected at the hotel which was the chief source of the Chicago epidemic, while the other two persons had not had contact with that hotel. All four of these strains produced a higher pathogenic index than any of the other strains studied. We wish to call special attention to the fact that two of these strains came from symptomless carriers, an illustration of the difference in the response of individual human hosts to highly pathogenic strains.

The last two strains recorded in Table II were sent to us as culture cysts by Mr. CLIFFORD DOBELL of London. (For his studies of these strains see DOBELL, 1931). The first strain, N. S. R., had been obtained from a naturally infected macacus monkey 8 years ago and had been cultured and passed through two other macaques and into culture again. Its infectivity for kittens had never been tested. In our series of kittens it produced a low percentage of infections and the lowest pathogenic index we have yet obtained. The second Dobell strain, D. M. R., had been obtained in culture $10\frac{1}{2}$ years ago by Dr. J. DRBOHLAV

in London from a human case of acute amoebic dysentery. This strain had successfully infected a kitten inoculated by DRBOHLAV with the eighth subculture but DOBELL had been unsuccessful in inoculating seven kittens with the 26th to 46th subcultures, and two other kittens $2\frac{1}{2}$ and $5\frac{1}{2}$ years respectively after isolation of the strain. He had likewise been unsuccessful in infecting kittens with four substrains of this strain after one monkey passage, using one kitten for each of the tests. One of these substrains had been passed through a second monkey and had then been in cultivation continuously for $5\frac{1}{2}$ years before it was sent to us. It will be seen that in our series of inoculations this strain infected six out of twenty kittens (30 per cent.) and that the pathogenic index (0.92) was nearly as high as that of the more actively pathogenic strains from Tennessee. The various kitten experiments performed with this strain emphasize the importance of using a reasonably large series of animals in determining the infectivity of a given strain of *E. histolytica*, in order to avoid false conclusions due to differences in the response of individual kittens. Of course it is possible that our kittens were more uniformly susceptible to infection with *E. histolytica* than are the kittens of London, and we hope that this point can be cleared up by further experiments in that city. Another possible factor in our success with this strain as well as with other strains is the inoculation of kittens directly into the ileum instead of by rectum as was done by DOBELL. Early in our work we found that ileum inoculation in kittens produced a higher incidence of infections than rectal inoculations, although it seemed to make no difference in the severity of the lesions in kittens which became infected.

The results of these experiments indicate, we believe, that, by the use of a large enough series of animals and by the employment of uniform and favourable methods of technique, most strains of *E. histolytica* from man and some at least from lower animals can be demonstrated to possess some degree of pathogenicity for kittens, furthermore that the differences between strains can be measured with some degree of accuracy, and that, as far as our observations go, the pathogenic index of a given strain remains fairly constant over a long period of time in artificial cultivation.

The influence of the accompanying bacteria on the pathogenic activity of *E. histolytica* is a point which must be considered. In direct kitten to kitten transfer, BAETJER and SELLARDS (1914a) found that the prepatent (incubation) period of the infection became progressively shorter, and from bacteriological studies they concluded that this probably was due to the transfer of virulent streptococci which secondarily invaded the amoebic lesions.

CLEVELAND and SANDERS (1930), in a study of amoebic liver abscesses which they produced in cats by direct injection of cultures of *E. histolytica* into the liver, found that bacteria capable of doing some damage to the liver must accompany the amoebae in order to produce an amoebic abscess, and that the bacteria in the amoeba culture lost this power after prolonged cultivation but regained it after repeated animal passage. In studying intestinal amoebic infections in

kittens they also obtained an increase in the percentage of successful injections by repeated animal passage, and concluded that this result was probably due to the increased virulence of the accompanying bacteria. Secondary bacterial infection often can be demonstrated in sections of the colon in severe amoebic lesions. Usually the bacteria are located more superficially than the amoebae in the tissues, but where deep lesions occur the bacteria are often found in the lymph vessels of the submucosa or even of the serosa and occasionally the amoebae have disappeared entirely from the lesions (MELENEY and FRYE, 1934). It has also been found by DOBELL and LAIDLAW (1926) and CLEVELAND and SANDERS (1930a) that in culture *E. histolytica* grows well with certain types of bacteria but poorly or not at all with others. We have found, however (FRYE and MELENEY, 1933) that when culture amoebae of a highly pathogenic strain are washed free from most of their own bacteria and are then mixed with bacteria accompanying a mildly pathogenic strain, or *vice versa*, no measurable change in the pathogenic index of either strain of amoeba for kittens is produced. The differences in pathogenic activity of different strains of amoebae appears, therefore, to be a characteristic of the amoebae themselves rather than of the bacteria which accompany them.

THE POSSIBILITY OF CHANGES IN THE PATHOGENICITY OF INDIVIDUAL STRAINS OF *E. histolytica*.

It is well known that certain bacteria lose their virulence after periods of artificial cultivation and that the virulence may be regained by serial passage through animals. In recent years this phenomenon has been ascribed to bacterial dissociation, the so-called rough strains being avirulent and the smooth strains virulent. Either strain can apparently originate from the other and both usually exist together in cultures. The preponderance of one strain over the other seems to determine the degree of virulence of the organism. Whether a comparable condition exists in the case of *E. histolytica* we do not know. Some investigators have found that prolonged cultivation or cultivation on media containing rice flour or rice starch apparently reduced or destroyed the pathogenicity, while others, including ourselves, have not found such a change (see Table I). Most of the observations in which a loss of pathogenicity has been found have been based upon such a small number of animals that the resistance of individual animals might easily account for the results obtained.

The evidence that strains originally possessing a low degree of pathogenicity can be made more pathogenic by repeated animal passage is also meagre. It is apparently true that direct animal to animal transfer of dysenteric discharges containing amoebae produces a higher incidence of infection than the use of culture amoebae, but in such a procedure one is dealing with active tissue-invasive amoebae, many of which have been feeding upon red blood cells and tissue fluids, and these amoebae may be more active in motility and metabolism

than the same strain would be in culture. We have attempted to step up the virulence of one of our less pathogenic strains by serial passage through kittens and dogs, but so far we have been unsuccessful because the first kitten to kitten transfer has always failed, and we have had the same experience with the one dog to dog transfer which we have been able to attempt.

It is obvious that any convincing evidence as to the possibility that strains of *E. histolytica* possessing a low degree of pathogenicity can become highly pathogenic, or that a highly pathogenic strain can lose its pathogenicity, must be based upon very carefully controlled experiments in a large series of animals.

DISCUSSION.

There is at least one other good illustration among the protozoa of man of the existence of strains of a single species differing in pathogenicity. BOYD, STRATMAN-THOMAS and KITCHEN (1935) have found strains of *Plasmodium vivax* which are of no value in the therapy of paresis because they fail to produce heavy infections, whereas other strains give rise to a typical course of tertian malarial fever.

A possible explanation of the present differences between strains of *E. histolytica* may be based upon historical and geographical considerations. We know that amoebae morphologically similar to *E. histolytica* exist naturally in frogs, snakes and monkeys, and the monkey amoeba has been conceded by most protozoologists to be *E. histolytica* itself. Man undoubtedly has harboured this parasite since prehistoric times and it is reasonable to suppose that various ethnological groups have become more or less adjusted to the parasite and the parasite to the host. The more pathogenic strains would seem to have existed under conditions of diet, climate and host resistance where they have been encouraged to attack the body tissues with greater avidity than the less pathogenic strains. Racial groups more or less isolated in the past, and still somewhat isolated as in the hill communities of Tennessee, would be likely to harbour closely related strains. The introduction of more active strains, like those native to the tropics, into communities in the temperate zones, and the introduction of people from the temperate zones into the tropics, where they are adjusted neither to the climate nor to the parasites, would give rise to more active evidence of amoebic infection than is usual among those groups of people. For example, it is easily possible, if not probable, that the Chicago epidemic had its principal origin in employees of the hotel who harboured highly pathogenic strains acquired in the tropics and who started the water contamination which ultimately led to a high degree of pollution. (See References—Amebiasis outbreak in Chicago.) Likewise it is a notorious fact that Europeans and Americans who travel or reside in certain tropical regions usually suffer more severely from acute amoebic dysentery and liver abscess than do the natives of those regions. On the other hand, BRUMPT (1928) has reported that there are areas in tropical

South America, for example the city of Rio de Janeiro, where the incidence of *E. histolytica* (his *E. dispar*) is high but where amoebic dysentery is uncommon. It is reasonable to assume that the strains imported into those areas with European immigrants have been the less pathogenic ones and that conditions such as exist in Africa, India, and China for the dissemination of the more pathogenic strains have not existed in these centres in South America. It would be dangerous, however, to assume on the basis of present evidence that the low pathogenic index of certain strains of *E. histolytica* is a stable phenomenon and that it is not possible for such strains to become highly pathogenic under favourable conditions of climate, diet, dosage or rapid transmission. Furthermore, because of the wide variation in the response of individuals to any given strain, every person known to harbour *E. histolytica* should be treated until the parasite is permanently eradicated.

SUMMARY.

The variations in the clinical picture of infections with *E. histolytica* appear to depend first upon variations in the resistance of individual hosts and secondly upon variations in the pathogenic activity of different strains of the amoeba. Both of these factors can be demonstrated experimentally in kittens if a large enough number of animals is used and if uniform conditions of experiment are employed. There is evidence that strains of a similar degree of pathogenic activity predominate in certain population groups where conditions favour the limitation of these strains to the groups concerned and the exclusion of other strains. Travel and the development of cosmopolitan populations tend to introduce other strains which may possess a different degree of pathogenicity. The pathogenic index of a given strain seems to be a fairly stable condition in the strains we have studied from this point of view. Prolonged cultivation in artificial media has not decreased the pathogenic index. Attempts to raise the pathogenic index have so far not been successful, although further attempts are necessary before conclusions can be drawn. In medical practice, however, any strain of *E. histolytica* should be considered dangerous and should be eradicated by treatment.

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A CONTROLLED EXPERIMENT IN THE TREATMENT OF MALARIA WITH ATEBRIN-MUSONATE BY INJECTION.*

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In March, 1935, it became apparent from routine entomological investigations that an epidemic of malaria might be expected to develop in Nairobi. In view of the publicity given in the lay press to the results to be anticipated from the use of atabrin-musonate, it was decided to test the efficacy of that preparation in case a severe and widespread outbreak should occur. Fortunately, however, towards the end of May there was a steady decline in the breeding of mosquitoes and the incidence of malaria fell off rapidly, thus relieving the medical authorities of grave anxiety occasioned by the heavy incidence of serious cases amongst those which did occur.

The lay press, in giving prominence to conditions prevailing in Ceylon during the recent epidemic, gave highly optimistic reports as to the results obtained with atabrin-musonate, reports which left in the mind of the public the impression that so far as malaria was concerned, the long looked-for "*therapia sterilisans magna*" had been discovered at last. Such unqualified optimism is unfortunate in its effect on the public when it appears in a daily newspaper; but it may be more harmful still when indulged in by writers in professional journals. There is a distressing tendency for new remedies to be hailed with an enthusiasm which has subsequently to be extensively qualified, as was the case first with plasmoquine and later with atabrin. MANSON-BAHR wrote less than a year ago (1934): "It is possible to exterminate this parasite [*P. falciparum*] in the blood with adequate doses of atabrin, with quinine perhaps as an adjunct, in a way never formerly anticipated; it is possible to prevent entirely the recurrence of relapses. . . . It is possible that in cases adequately treated with atabrin, blackwater fever will not develop." This series of statements would hardly be accepted by many workers of experience to-day and before the millions

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of quinine-treated cases of the past are discounted so readily, many thousands of patients must be treated and followed up all over the world. The wholesale use in an epidemic of an almost untried preparation may be not only futile but positively dangerous and evidence is given below in support of the latter contention.

ATEBRIN-MUSONATE.

Atebrin-musonate is a yellow crystalline powder readily soluble in water and described as being an atebrin salt of musonic acid which was evolved by the makers purely for use by the parenteral route. The drug is obtainable in glass ampoules containing 0.375 gramme which is equivalent to 0.3 gramme of atebrin and is prepared for use by injecting 10 c.c. of distilled water into the ampoule after first cutting off the top. It may be injected either intramuscularly or intravenously and in the former case the makers recommend a dose of the whole ampoule, while for intravenous use a third of the solution is said to be the maximum which should be given. The cost in Kenya worked out at 2s. 6d. per ampoule and since it was not possible to obtain more than a small quantity these maximum doses were not exceeded, although there was every indication that more could have been tolerated without passing the limit of safety.

Atebrin-musonate in Ceylon.

Subsequent to the commencement of the clinical experiment which forms the subject of this paper, a report was published by BLAZE and SIMEONS (1935) upon a series of twenty-one selected cases of malaria treated with atebrin-musonate, and their paper was made the subject of an editorial in the same issue of the *Indian Medical Gazette*. Since at the time of writing this is, so far as can be ascertained, the only work which has been published on the subject, it may be well to summarise shortly the results given:—

Of the twenty-one cases, three were also given atebrin orally and will not therefore be considered.

Three cases received a single injection (presumably intramuscular 0.375 gramme). In two, both benign tertian infections, parasites persisted for 1 and 2 days respectively and for 3 days in the third, a mixed infection of benign tertian and malignant tertian. In all three cases the temperature fell promptly to normal and did not rise again.

In nine cases, two injections were given (again presumably intramuscular 0.375 gramme). All of these were infections with *P. falciparum* except one which was with *P. vivax*. In each case the temperature was finally controlled by the second injection and parasites persisted on an average $1\frac{1}{2}$ days after the second injection. It should be noted however that the reason for the second injection was the reappearance of parasites after an interval of some days. In two cases the first injection was specified as being intravenous 0.125 gramme.

Of the remaining six cases two were benign tertian, three malignant tertian and one a mixed infection of these two forms. Again the injections were not necessarily given on successive days, but rather as and when rings were found to have reappeared in the peripheral blood, or when there was a return of fever. In all except one case, the fever was finally controlled by the third injection. Creasents were noted as occurring at some stage in 50 per cent. of the malignant tertian cases.

In discussing the advantages of giving atebtrin parenterally rather than orally, BLAZE and SIMEONS suggest that when given by the mouth the drug is absorbed, carried to the liver and then excreted with the bile into the duodenum. It is assumed that this process of absorption, excretion, and re-absorption continues until the liver reaches a hypothetical state of saturation, whereupon atebtrin passes into the general circulation and is able to exert its therapeutic effect upon the patient. Thus the slowness with which atebtrin has been said to control malaria is explained and the adoption of parenteral administration as a routine is justified. The work of HECHT, TROPP and WEISE is quoted as supporting the theory.

In the *Indian Medical Gazette* Editorial (1935), it is agreed that parenteral atebtrin-musonate may prove to be a more powerful anti-plasmodial agent than oral atebtrin-dihydrochloride but the theory of preliminary hepatic saturation is questioned and it is pointed out that by no means all workers have noted the latent period before oral atebtrin influences malarial attacks.

PETER (1935) also challenges the theory of BLAZE and SIMEONS though he agrees that metabolic processes must undoubtedly play a part in the action of the drug. He notes a case in which a patient when first treated showed marked skin staining and relapsed, while following a second course, after which no discoloration of the skin was recorded, he appeared to be permanently cured. It is pointed out that atebtrin is a dyestuff and that the staining of the skin implies that much of the drug is lost to the general circulation and that more still may be locked up in staining the mucous lining of the digestive tract.

The theories of PETER, though so far unconfirmed by experiment, would appear to be more tenable than that of BLAZE and SIMEONS.

Since all but one of the patients in the series described in the present paper were Africans, it was not possible to make any observations upon the frequency with which skin staining occurred, or upon the relation of that phenomenon to the effects of treatment.

METHODS ADOPTED.

In order to have a standard of comparison which would eliminate as far as possible errors arising out of the variability in virulence of different strains of parasites, a complete series of control cases was included. Patients were numbered according to their order of admission to hospital and were all treated in one ward. The *odd numbers* were placed on one side and treated with atebtrin-musonate and the *even numbers* opposite to them acted as controls.

The even numbers were treated according to no hard and fast rule but in the light of previous local experience received quinine by the mouth or by injection as the severity of the case indicated. All of them received plasmoquine 0.01 gramme t.i.d. for five days. The dose of quinine given was 10 grains of the dihydrochloride t.i.d. in solution.

There was no local knowledge as to dosage and the limited amount of atebtrin-musonate available did not allow of an extensive test of a variety of doses, so that it was decided to adhere to the recommendations of the makers, *viz.* 0.125 gramme intravenously or 0.375 gramme intramuscularly, although as noted above, experience showed that larger doses might have been given with safety.

All cases, whether on atebtrin or quinine, received calomel and salts on admission and were given free draughts of sodium bicarbonate solution. The only other drugs used were aspirin for headache and pain in the limbs, and a simple iron and strychnine tonic in most cases.

A daily thin smear and thick drop were made from each patient and an attempt was made to continue the series for 10 days, though it was not possible to do this in the majority of cases, since when a sense of well-being returns native patients are loath to remain in hospital.

All the slides were examined and the parasites counted by one observer (R.P.C.), the stain used was Leishman's and the number of parasites in fifty fields of the thin smear was counted. It is recognized that this method is open to certain objections, but as the object of the investigation was to compare two systems of treatment and since the personal factor remained constant throughout, the objections are not very important. An endeavour was made to minimize one objection by establishing a sort of mental standard of density and then ignoring fields either markedly thicker or thinner than this standard, thus as far as possible fifty similar fields were used in making each computation.

Search was not limited to fifty fields and in all cases the thick drop was examined first to determine the presence or absence of parasites. Even when no parasites were seen in the thick drop, the thin smear was examined for any abnormality in the erythrocytes if such had been previously noted in that particular case.

All the parasites were *P. falciparum* except in two cases, Case 30, a mixed infection with *P. vivax* and *P. falciparum*, and Case 57 which showed *P. malariae* only. The tables give the results of these examinations and the figures in each case are to be taken as giving the numbers of parasites in fifty fields of a thin smear. A negative result was only recorded when the whole of the thick drop had been searched in vain.

Source of Patients.

The tribe and district from which a malarial subject is derived may have an important bearing upon his reaction to treatment, since he may have lived in an area where the disease is absent, endemic or hyperendemic. Although in one column of the tables an indication is given as to whether the attack was an initial one or not, too much importance must not be attached to this information because of the inability of most natives to remember what may have been minor illnesses in childhood.

Though situated close to native reserves, Nairobi does not derive from those reserves more than a certain percentage of its African population: it comes also from outlying districts whence labour is recruited for a variety of purposes. Fifty-five of the total of 68 patients were derived from a tribe, the Kikuyu, amongst whom malaria is not endemic. This represents 82 per cent. of the cases and so the chances are that when 43 (63 per cent.) of the patients gave a history of having had no previous attack, most of them were fairly near the truth. These facts, combined with the severity of many of the attacks, confirm the view that there was real danger of an extensive epidemic.

Patients had been ill on the average for 4 days prior to the time of admission to hospital. The time in each case is noted in the third column of Tables I and II.

LABORATORY FINDINGS.

(a) Asexual Forms.

In the quinine control cases asexual forms persisted on an average for 1.32 days from the time when treatment began. In patients receiving 0.125 gramme of atebirin-musonate intravenously this period was extended to 6.75 days ; for those who were given three intravenous injections of the same dose, to 3.55 days ; and where three intramuscular injections of 0.375 gramme each were given, to 1.75 days. There is however one further point of interest, namely, that in the atebirin series there appeared to be a tendency for the numbers of parasites in the finger blood to rise more markedly after the first injection than was noted in the quinine controls. If this finding is confirmed by future work, it would appear to indicate that atebirin-musonate tends to force the parasites out of internal organs into the general circulation.

One point is quite definite so far as this small series is concerned, namely, that parasites persist in the peripheral blood rather longer with intramuscular atebirin-musonate than they do with quinine and plasmoquine.

(b) Sexual Forms.

In the quinine and plasmoquine series there were 10 cases or 29.4 per cent. which at some time showed crescents, though in only one case did these persist up till the time of discharge from hospital. In the atebirin-musonate series there were 19 cases or 55.8 per cent. showing crescents, which persisted in 12 until discharged from hospital or, as happened in two cases, until eliminated by plasmoquine treatment. Crescents appeared on an average just over 8 days from the onset of symptoms and the time was almost the same for both series of cases. This is rather shorter than the 10 days given by THOMSON and ROBERTSON (1935) and the 11 days given by GARNHAM (1931).

One noteworthy difference between the two series is that those patients on quinine and plasmoquine not only developed crescents less frequently but when they did produce them they did so in very much smaller numbers.

While the number of cases examined is small, yet consideration of these figures leads to the view that the inefficiently or insufficiently treated patient can be a great potential danger to himself and to the community at large. If parasites of both forms persist in the blood stream after the patient feels well, injudicious exertion or some other factor may determine the onset of pernicious symptoms, while by reason of the large numbers of presumably mosquito-infective forms in his blood after he has apparently recovered normal health he is a potential danger to his fellows. It still remains to be seen whether these gametocytes are in fact infective to mosquitoes, though nothing was noted in their microscopical appearance to indicate that they were not. A dangerous sense of security may be derived from the use of such a drug as atebirin-musonate given alone in cases of moderate severity on account of its admirable capacity for reducing the temperature and conferring a sense of well-being without apparently affecting the parasites very much ; *vide* Cases 1, 3, 5 and 7 in Table II.

[illegible]

[illegible]

Heavy horizontal line = Duration of fever after admission; T.D. = Thick drop; B.T. = Benign tertian; S.T. = Sub-tertian; + = parasites found; — = No parasites found. All parasites found were *P. falciparum* except Case 30, a mixed infection of *P. falciparum* and *P. malariae*. Figures in each case = Numbers of parasites in 50 fields of a thin smear (Leishman stain). Inject. = An injection of quinine.

TABLE II.—ATEBRIN-MUSONATE CASES.

Number of Case	Number of attack	Duration (in days) of symptoms before admission	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10	
			Asexual	Sexual	Asexual	Sexual	Asexual	Sexual	Asexual	Sexual	Asexual	Sexual	Asexual	Sexual	Asexual	Sexual	Asexual	Sexual	Asexual	Sexual	Asexual	Sexual
1	1st	4	91 I.V.		28		2		44		3		15		20		27	1	20	1	27	
3	1st	3	50 I.V.		21		4	15	5	16	—	30	—	33	—	—	—	—	—	54	4	23
5	1st	24	4 I.V.		152		86	1	13 I.V.		24 I.V.	3	2	6	—	6	—	5	—	15		
7	1st	3	1 I.V.	1	24		—		10	1	T.D. +		17	4	3	15	51	6	3	8		
9	1st	3	4 I.V.		25		—		—		—		—		—		—		—			
11	1st	3	4 I.V.		450		14 I.V.		87 I.V.		T.D. +		—		—	1	—	3	—	7		
13	4th	1	4 I.V.		56		14 I.V.	2	14 I.V.	T.D. +	—	2	—	T.D. +	—	T.D. +	—	T.D. +	—	T.D. +		
15	1st	3	4 I.V.		26		49 I.V.		10 I.V.		2	T.D. +	—		—	3	—	3	—	6		
17	1st	4	200 I.V.		123 I.V.		130 I.V.	1	7	2	—	17	—	64	—	—	182	275	—	98		
			1 P.	4	—	1	—		—		—		—		—		—	—	—	—		
19	3rd	3	4 I.V.		48 I.V.		14 I.V.	4	—	7	—	12	—	17	—	19	—	—	—	—		
21	2nd	3	250 I.V.		400 I.V.		62 I.V.		45		—	T.D. +	—		—	—	—	1	—	—		
23	2nd	3	4 I.V.		74 I.V.		39 I.V.	1	8		—		—		—	—	—	—	—	—		
25	1st	7	4 I.V.		13 I.V.		4 I.V.	2	—	1	—	1	—	1	—	—	—	T.D. +	—	—		
27	died	1	1 M.		T.D. +		—															
29	1st	6	24 I.M.		1 M.		58 I.M.		—		—		—		—		—	—	—	—		
31	1st	5	530 I.M.		1,500 I.M.		29 I.M.		T.D. +		1	1	—		—		—	—	—	—		

CLINICAL RESULTS.

(a) A Single Intravenous Injection.

As will be seen from Table II the first patients were given only a single intravenous injection of 0.125 gramme of atebirin-musonate. It was not found to be necessary to administer the dose very slowly; in general half a minute was taken over the injection. There was no tendency for shock or any other untoward symptom to develop either during or after the injection, nor was there any indication of venous thrombosis supervening. On one occasion a small quantity of solution was injected into the subcutaneous tissue outside the vein but there was no painful or inflammatory reaction.

Eight patients were treated with a single injection; in four the temperature fell to normal in the first 24 hours, and of these four one only had a return of fever. In this group of cases the fall of temperature was accompanied by an apparent return to normal health although asexual parasites were still present in considerable numbers in the finger blood. In view of this latter fact, patients after the first four were given two more injections as indicated in Table II. The third case, No. 5 in Table II had a return of headache and began vomiting on the 4th day; he was given a second and third injection on that account, and all symptoms cleared up at once while parasites disappeared on the 6th day. Cases 1, 3, 7, and 9, which were all patients with typical sharp first attacks of malignant tertian malaria who never had more than one injection, lost all fever and symptoms in 24 hours; though kept under observation for 10, 10, 9 and 9 days respectively they still had parasites, both sexual and asexual, in the peripheral blood (except Case 9) when they left hospital. If the first five quinine control cases are compared with these atebirin cases, it will be seen that while fever and malaise persisted, asexual parasites were not found, with one exception after the 2nd day and that in that exception they were found only in the thick drop on the 5th day. Two of them showed crescents which, however, rapidly disappeared. The relative infrequency and rapid elimination of crescents in the quinine cases is, of course, attributable to the concurrent administration of plasmoquine; but the persistence of asexual forms with the heavy gametocyte rate in the atebirin-musonate cases is too nearly constant to be fortuitous, while its association with complete freedom from fever and with an apparent return to normal health, is not easily understood in patients who had no acquired immunity to the disease. Attention has already been drawn to the dangerous sense of security that might be derived from the results of treatment along these lines unless a daily blood examination were made.

(b) Three Intravenous Injections.

Nine cases received three intravenous injections of 0.125 gramme, namely Cases 5, and 11 to 25 inclusive. Case 5 has already been considered and of the remaining eight, in only one, Case 17, were asexual forms found in the thin

smear later than the day following the third injection. In Case 17, a single ring was found in the 50 fields of the thin smear on the 8th day after the last injection and this case was also remarkable for the appearance of very large numbers of crescents first seen on the 6th day from the onset of symptoms and persisting until the end of a 5 days course of plasmoquine. In this group, crescents appeared in every case and in seven of the ten persisted until the 8th day or longer. Of the corresponding quinine controls, Cases 10 to 26 inclusive, only three showed crescents, which in one case were still found in the thick drop when the patient was discharged on the 7th day.

(c) *Three Intramuscular Injections.*

The remaining cases, Cases 29 to 67 inclusive were treated with three intramuscular injections on three successive days, the dose given was 0.375 gramme, and since Case 61 was a case of blackwater fever there were 19 cases in the series of which one, Case 57, was an infection with *P. malariae*. No. 27 was a fatal case of cerebral malaria and is discussed separately below.

In the 19 cases comprising this group the effect upon temperature and parasites may be summarized as follows. In 5 there was no further rise of temperature after the first injection, in 6 none after the second injection, in 6 none after the third, and in 2 the temperature remained up during the day following the third injection. In some of these cases the infections were very heavy and the patients were gravely ill. In 1 case the blood became negative and remained so after the first injection, in 6 after the second injection, in 8 after the third injection, in 3 cases asexual parasites were absent after 1 more day or 4 days in all, while in one case they persisted until the 5th day. In this series crescents appeared at one stage or another in 7 cases, and persisted for 7 days or longer in 4. Case 65, the only European included in the experiment, received a fourth injection and is discussed in detail below.

A comparison between these nineteen cases and the corresponding quinine controls is given below.

TABLE III.

	1st Day.	2nd Day.	3rd Day.	4th Day.	5th Day.
<i>Return to Normal Temperature :</i>					
Atebrin-musonate cases	5	6	6	2	
Quinine controls	2	7	7	3	
<i>Negative Blood Slides :</i>					
Atebrin-musonate cases	1	6	8	3	1
Quinine controls	4	6	5	4	

Table IV summarizes the findings for the whole experiment.

TABLE IV.

	Quinine Controls.	Atebrin-Musonate Cases.		
		One Intravenous Injection.	Three Intravenous Injections.	Three Intramuscular Injections.
Number of cases	34	4	9	19
Days of fever after beginning treatment	1.67	0	1.33	1.2
Persistence of asexual forms in days after beginning treatment	1.32	6.75	3.55	1.75
Percentage of cases showing crescents	29.4	75	100	35
Percentage of cases with crescents persisting till 7th day or longer	33.3	100	88	57.1

In a previous experiment, one of us (J. A. C., 1935) found in a series of 88 cases of sub-tertian malaria treated with atebrin by the mouth, that the average times required for the temperature to fall to normal and asexual parasites to be eliminated from the peripheral blood were 2.8 days and 3.0 days respectively. Thus atebrin-musonate intramuscularly for 3 days is more rapid in its action on the fever by 1.6 days, and in its action on the asexual parasites by 1.25 days.

Case 65.

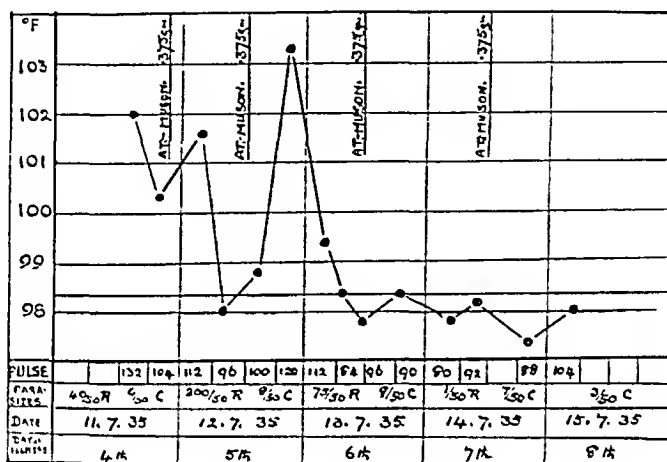
This was, as has been noted, the only European in the series and since the case presents one or two points of interest, it will be described in detail. The patient was a robust adult male, an aviator by profession, who had been treated for his first malarial attack two months previously. The said first attack was a severe one and was treated with oral atebrin 0.1 gramme t.i.d. for 5 days, followed by a second similar course after a week's interval. After a convalescence at sea level there was a relapse of moderate severity which was treated with oral atebrin t.i.d. for 7 days and plasmoquine simplex t.i.d. for 5 days after the atebrin. A week later, the patient was able to pass the severe medical examination required of commercial pilots and resumed flying. On June 16th he had a forced landing in an unhabited area and was obliged to walk with his passenger through the heat of the day for many miles on an empty stomach. This resulted in another relapse which he treated himself with atebrin t.i.d. for 5 days.

The attack under review commenced 3 days before admission to hospital with rigors, vomiting and headache.

Treatment began on July 11th with an intramuscular injection of atebrin-musonate. At that time, there were forty rings and six crescents in fifty fields. The next day the

temperature fell, another injection was given and later the temperature rose to 103.4°F . This, however, was the end of the fever and two more intramuscular injections were given. The chart shows the progress of the case. As indicated in Table II plasmoquine was given for 5 days after discharge from hospital in order to eliminate cresscents.

CASE 65.



On the day following discharge from hospital the patient felt better than he had done since the time of the original malarial attack and was able to pass his pilot's medical examination 7 days after the plasmoquine was stopped. He has remained free from relapse for 5 weeks.

Case 61.

This patient was a member of a tribe amongst whom malaria is hyperendemic and had fever with rigors for 3 days before admission. His urine was the colour of port wine but he had no temperature and his blood was negative. Since the haemoglobinuria was uncomplicated he was given no more drastic treatment than a brisk purge and copious saline draughts. Three intramuscular injections of atebirin-musonate were given and the patient made an uneventful recovery, the urine being free from haemoglobin 36 hours after treatment began.

DISCUSSION.

The questions to which it was hoped that this investigation would give preliminary answers were as follows:—1. Does the introduction of atebirin-musonate represent a definite advance in malaria therapy? 2. What is the best method of administration? 3. How does the cost of a course of atebirin-musonate compare with the cost of treatment by other methods?

1. Does the introduction of atebirin-musonate represent a definite advance in malaria therapy?

There appears to be no doubt that this question may be answered in the affirmative. In the first place it is not possible to rely wholly or even largely, upon any anti-malarial drug which can be given only by the mouth, at all events such is the case so far as East African malignant tertian malaria is concerned. Atebrin is now considered by a large percentage of malariologists to be a valuable

use in natives, but the slight inconvenience involved and lack of all unpleasant toxic symptoms, make it a strong rival to quinine and oral atebtrin for patients able to bear the expense.

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THE YELLOW FEVER IMMUNITY SURVEY OF NORTH, EAST AND SOUTH AFRICA.

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Since early in 1931 the International Health Division of the Rockefeller Foundation, with the co-operation of the governments concerned, has been carrying on an investigation of the geographical distribution of yellow fever immunity in man. For this purpose many specimens of blood serum have been collected and tested for their power to protect mice against yellow fever virus by the intraperitoneal protection test of SAWYER and LLOYD (1931). The principal objects of this survey are to delimit the areas of the world in which the disease has recently been present and may now exist and to contribute to the knowledge of its epidemiology.

This report is the fourth to be published by various authors on the distribution of yellow fever immunity in different regions of Africa. The greater part of West Africa was investigated by BEEUWKES and MAHAFFY (1934), and the remaining area was independently surveyed for the French Government by STEFANOPOULO, as published by BOYÉ (1933). A region including the French Cameroons, French Equatorial Africa, the Belgian Congo, and Angola was reported on by BEEUWKES, MAHAFFY, BURKE and PAUL (1934). This investigation of the northern, eastern, and southern parts of Africa and Spanish Guinea completes the general survey of the continent.

Additional information regarding two of the countries included in the regional surveys has been published. HEWER (1934), who had collected the sera from the Anglo-Egyptian Sudan, summarized and discussed the protection test results for that country. MOUCHET, VAN HOOFF, DUREN, FORNARA, CLAREBOUT, HENRY and HENRARD (1934), who had co-operated in the immunity survey of the Belgian Congo, presented further particulars regarding the conditions there.

In the survey here reported the blood sera were obtained for us by the health officials of the several countries. An exceptional opportunity for discussing the value of the survey and inviting the co-operation of representatives of the health services in its extension to the eastern and southern parts of Africa was afforded to one of the authors by the Cape Town Conference held in November, 1932, under the auspices of the Health Organization of the League of Nations. The Permanent Committee of the Office International d'Hygiène Publique, in

a letter from its president dated December 31st, 1930, had previously communicated its request that the Rockefeller Foundation undertake the technical management and organization of investigations of the areas of endemic yellow fever. These investigations were to be made by the powers concerned or with their consent and help.

The work in British colonies was facilitated by the co-operation and valued suggestions of Sir THOMAS STANTON, Chief Medical Adviser to the Secretary of State for the Colonies, and by the offer of Dr. G. M. FINDLAY of the Wellcome Bureau of Scientific Research to assist in arranging for the collection of sera and to receive the specimens in London and re-ship them to New York under suitable conditions. Dr. G. J. STEFANOPOULO of the Pasteur Institute in Paris assisted by obtaining specimens from the French colonies through local health officials, and by testing some of them for us in his laboratory by the methods used in this survey. The effective and prompt co-operation of many persons greatly hastened the completion of the general immunity survey of Africa and made it unnecessary for the International Health Division to plan and finance special expeditions for the collection of sera.

As in the surveys of BEEUWKES and his associates, the sera were collected as far as practicable in lots of twenty-five. When there was no reason to suspect the presence of immunes, the sera were collected from adults only, but elsewhere the aim was to secure twenty-five specimens from adults and twenty-five from children. Persons were selected for bleeding who were natives of the region and had never been away, but in certain instances to be mentioned it was discovered afterwards that donors of protective sera had been in distant places. The particulars regarding the donors were entered on printed forms and sent with the specimens. With a few exceptions, the sera were prepared in a government laboratory and shipped in sealed ampoules under refrigeration to New York, where they were tested in mice. A few shipments of whole blood were received in the vacuum syringes ('venules') in which the blood had been collected and were nevertheless usually satisfactorily examined. The method of testing the sera and reporting the results was that originally described by SAWYER and LLOYD (1931) except for two minor changes. The test had been made slightly less sensitive, or less likely to produce positive results, by increasing the strength of the virus-containing mouse-brain emulsion used in the serum-virus inoculations from 10 to 20 per cent. The results of tests in which only two of five or six mice survived were classed as negative instead of inconclusive as previously. To be considered protective a serum had to permit, as in the original method, the survival of all but one of five or six mice, or all of four, from the 4th to the 10th day after inoculation. Six highly susceptible mice were used for the test of each serum and for each control.

The results of the tests are presented in the Table and Maps I and II. For convenience in presenting and discussing the findings, the blood donors from 3 to 16 years of age were classified as children, and those over 16 years old

TABLE.

RESULTS OF TESTS OF SERA FOR PROTECTIVE POWER AGAINST YELLOW FEVER VIRUS.

Country.	Town or Locality.	Ages of Donors (Years).	Number of Specimens.	Sera Giving Protection.		Youngest Donor of Protective Serum (Age in Years).
				Number.	Per cent.	
Morocco	Casablanca	17-45	24	0	0	
	Fez	22-45	17	0	0	
	Rabat	22-70	45	0	0	
Algeria	Oran	Adults	28	0	0	
Tunisia	Tunis	Adults	25	0	0	
Egypt	Assuan	17-65	28	0	0	
	Assyut	17-60	48	2	4.2	20
	Luxor	17-48	51	0	0	
Anglo-Egyptian Sudan	Mansura	6-16	15	0	0	
		17-68	95	1	1.1	22
	Amadi	4-16	17	0	0	
		17-60	19	2	11	20
	Dilling	3-16	15	0	0	
		17-80	30	7	23	30
	El Fasher	4-16	18	0	0	
		17-50	20	9	45	25
	El Obeid	5-16	20	0	0	
		17-70	32	0	0	
	Geneina	3-16	13	0	0	
		17-47	25	2	8	19
	Juba	3-16	22	3	14	7
		17-55	29	2	7	
	Khartoum	17-46	32	0	0	
	Li Rangu	3-16	15	0	0	
		17-40	16	4	25	28
	Malakal	2-16	23	0	0	
		17-70	27	1	3.7	18
	Rumbek	3-16	26	1	3.8	11
		17-60	35	16	46	
	Wau	4-16	24	3	13	6
		17-60	31	8	26	
	Yirol	3-16	19	0	0	
	Yubo	3-16	17	0	0	
		17-55	20	5	25	30
	Zalingi	3-16	14	0	0	
		17-48	16	2	13	27
Ethiopia	Addis Ababa	19-60	27	0	0	
British Somaliland	Berbera	16-45	10	0	0	
	Burao	18-62	19	0	0	
	Hargeisa	20-50	15	0	0	
Uganda	Ajumani	8-16	17	0	0	
		17-50	29	0	0	

TABLE—(Contd.).

Country.	Town or Locality.	Ages of Donors (Years).	Number of Specimens.	Sera Giving Protection.		Youngest Donor of Protective Serum (Age in Years).
				Number.	Per cent.	
Uganda	Aringa	7-15	20	0	0	25
		20-55	31	0	0	
	Arua, Maracha, Vurra Group	8-11	25	0	0	
		19-60	67	4	6	
	Fort Portal & Toro District	18-40	21	2	10	30
	Gulu	8-16	21	0	0	Adult
		20-60	30	0	0	
	Kaiso, Kibero, Kibanda, Buhuka	16-50	19	1	5.3	
	Kigezi, Kabale and extreme West	17-21	24	2	8	
	Kitgum	8-12	20	0	0	18
		17-45	30	3	10	25
	Lira	8-12	24	0	0	25
		18-50	30	1	3.3	
	Masindi	8-13	25	1	4	
		20-50	25	0	0	
	Moyo	8-10	19	0	0	30
		18-35	32	1	3.1	
	Mugwere, Mugishu, Musoga, Munyuli Tribes	6-16	25	0	0	
		20-50	26	1	3.8	
Kenya	Fort Hall District	17-40	26	0	0	25
	Kakamega	Adults	55	0	0	
	Kisii	17-35	25	1	4.0	
	Kisumu	Over 16	42	0	0	
Tanganyika	Bukoba and Muhamba	20-40	23	0	0	20
	Dar-es-Salaam	20-40	25	0	0	
	Kigoma	20-45	25	0	0	
	Mpwapwa	18-50	25	0	0	
	Mwanza	19-40	25	1	4.0	
	Tabora	20-46	23	0	0	
	Tinde	20-40	23	0	0	
	Uzinza and Urima	20-40	25	0	0	
Zanzibar	Zanzibar	15-45	62	0	0	

TABLE—(Contd.).

Country.	Town or Locality.	Ages of Donors (Years).	Number of Specimens.	Sera Giving Protection.		Youngest Donor of Protective Serum (Age in Years).
				Number.	Per cent.	
Northern Rhodesia	Barotseland and Livingstone	18-36	21	0	0	24
	East Luangwa	17-46	25	1	4.0	
	Northwest Area	18-50	25	0	0	
	West Luangwa	18-42	25	1	4.0	30
Southern Rhodesia	Mtoko District	16-60	22	0	0	
Madagascar	Antananarivo	Adults	20	0	0	
Bechuanaland Protectorate	Serowe	19-56	13	0	0	
Union of South Africa	Cape Town	19-30	24	0	0	
	Durban	20-74	25	0	0	
	Tongaland	16-50	42	0	0	
Spanish Guinea	Oveng	18-60	45	8	18	18
	Machinda	18-60	36	1	2.8	30
	Bimbongo and Elobey	20-65	10	0	0	13*
	Total Spanish Guinea (adults)	18-65	91	9	10	

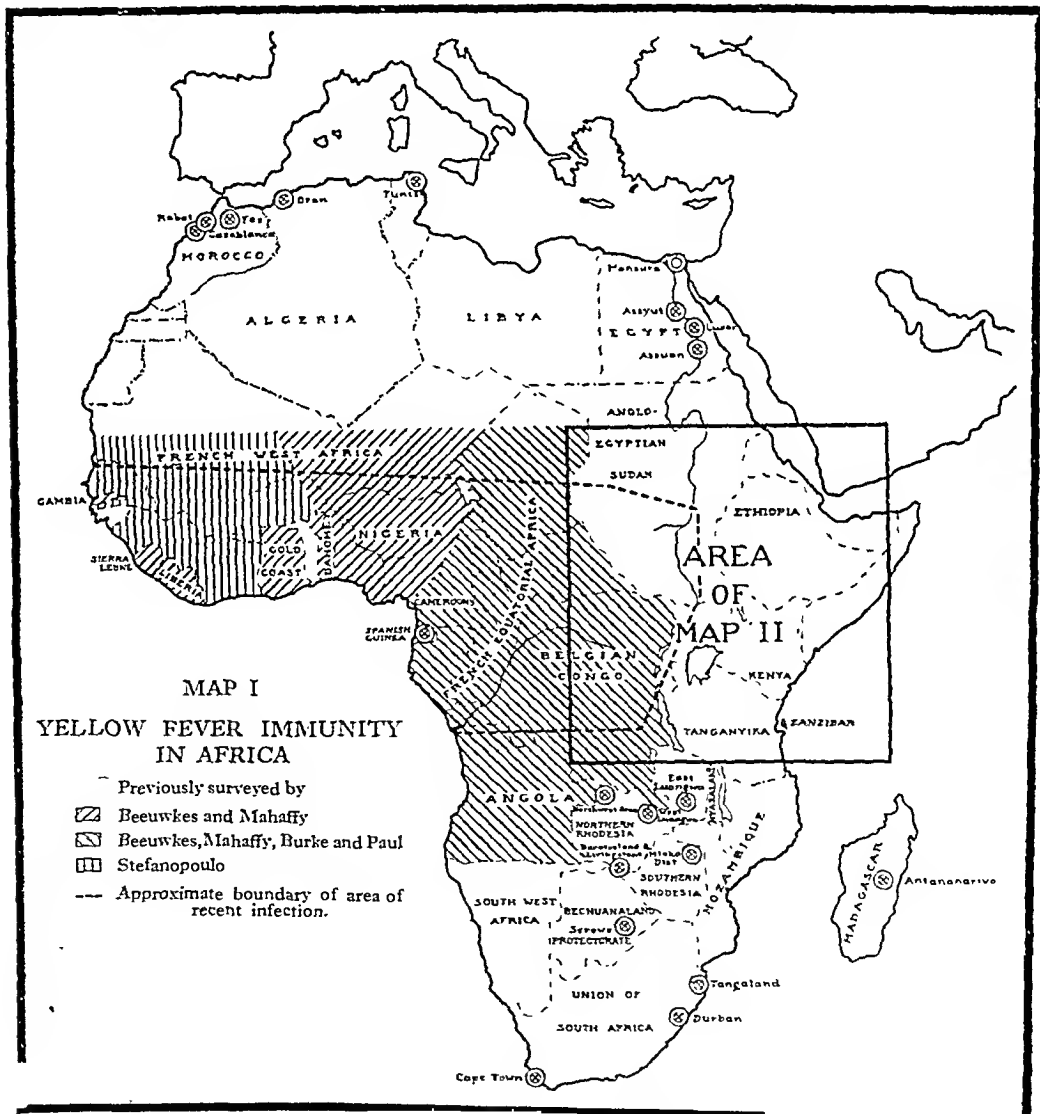
*Among five additional sera from children of Bimbongo, there was one protective serum from a donor 13 years old.

as adults. A few persons 15 or 16 years old were sometimes grouped with the adults when no special collection from children was made. Groups containing less than ten donors, and those for which ages or exact locations were not obtained, were omitted from the table and the maps. There were no protective sera among those dropped for these reasons, except in one specially mentioned case in Spanish Guinea.

In the Table are given for each locality the age groups of the donors, the number of specimens satisfactorily examined, the number of protective sera, the percentage of protective sera among all sera satisfactorily examined, and the age of the youngest donor yielding a protective serum. In Maps I and II the percentages of protective sera among children and adults are graphically presented for each locality. In Map I are shown also the areas covered by the previously published surveys and the approximate boundary of the part of Africa which has undergone infection during the lifetime of the present generation. This vast recently-infected region, which probably corresponds in general with the endemic area of yellow fever in the Eastern Hemisphere, extends into the

territory covered by this survey. From the data previously published and those now presented, it is apparent that the infection is irregularly distributed within the endemic area and may be absent from parts of it for considerable periods of time. The absence of immunity in some places from all the donors examined, and in others from the children only, is evidence of this.

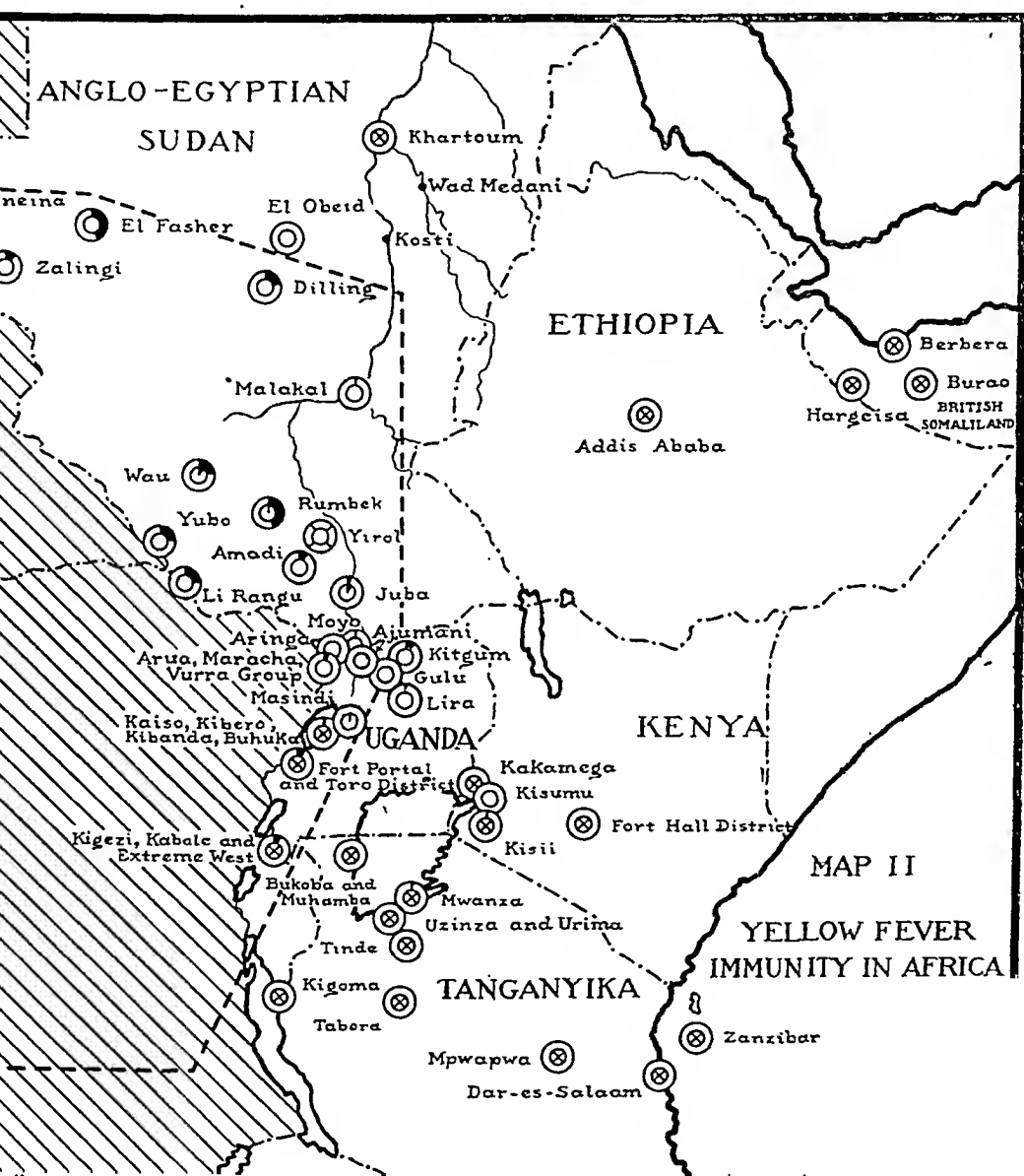
The relation of the geographical distribution of the clinically recognized yellow fever of the past to the area of recent immunity has been discussed for



MAP 1.—DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN AFRICA.

Outer circles represent adults. Inner circles represent children. Black sectors indicate the proportion with protective sera. Circles crossed with lines signify that no tests were made.

ca as a whole in another publication (SAWYER, 1935). All the historic breaks of yellow fever in Africa occurred within the area of recent immunity defined in Map I except some epidemics further south on the coast of Angola (MAS MORA, 1933) and two invasions of Morocco. Yellow fever was never reported from the interior east of Nigeria, even within the area of recent immunity, except for the one case recently observed at Wau in the Anglo-Egyptian Sudan



2.—DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN THE CENTRAL PART OF EAST AFRICA.
Symbols as in Map 1.

(HEWER, 1934). Within the area of this investigation Morocco is the only country which has a clear history of the presence of yellow fever at any time prior to this study (AUGUSTIN, 1909). In 1804 the disease was brought from Malaga to Penon de Velez, a fortress on a small island in the Mediterranean south-east of Ceuta, and became epidemic there. The one recorded introduction to the mainland occurred at Tangier in 1881, but there were only a few sporadic cases and no deaths.

MOROCCO, ALGERIA AND TUNISIA.

Sera were obtained from Casablanca, Fez and Rabat in Morocco in October, 1933, through the kindness of Dr. G. J. STEFANOPOULO and Dr. GAUD, Inspector to the Department of Health and Public Hygiene of Morocco. As is shown in the Table none of the 86 sera protected mice against yellow fever virus. Fifteen additional sera from adult natives of the same cities were examined for us by Dr. STEFANOPOULO and the results were likewise negative.

From Oran in Algeria and Tunis in Tunisia, Dr. STEFANOPOULO obtained 53 specimens from adult natives in 1934 and examined them for us at the Pasteur Institute in Paris. None of the sera gave protection.

The results for these three countries, together with the yellow fever history of the region, were accepted as evidence that the disease was probably not present in the region north of the Sahara Desert.

EGYPT.

The specimens from Egypt were obtained through the kindness of Dr. M. SHAHIN, Under-Secretary of State for Public Health. The donors were bled between 29th November and 28th December, 1932. They were native Egyptians who had lived all their lives in their present localities. As shown in the Table there were 3 protective sera among 237 collected in 4 cities. The protective sera were 1·3 per cent. of the total number. One of two protective sera from Assyut gave protection also on retest, and a second specimen obtained six months later from the same donor at first gave an inconclusive result (partial protection), but gave protection on retest. This donor's serum seems to have a persistent but low protective power, which would ordinarily be accepted as evidence of previous infection with yellow fever virus. The other protective serum from Assyut was of doubtful significance as its protective power was weak and transient. The first result was inconclusive, but there was protection on retest. A specimen obtained six months later was entirely without protective power in two tests. The protective serum from Mansura could not be retested as there was not enough serum, and the donor had died of pellagra when a second specimen was sought six months later.

The results show that Egypt has not been invaded in recent times and is outside the area of recent immunity. The extremely few protective sera may have been due to sporadic infection introduced by travellers from further south under conditions unfavourable to the spread of the disease, or possibly to an exceptional concentration of some non-specific factor in the blood.

ANGLO-EGYPTIAN SUDAN.

For the large number of well-selected sera from the Anglo-Egyptian Sudan we are indebted to Dr. O. F. H. ATKEY and Dr. E. D. PRIDIE, successive Directors of the Sudan Medical Service, to Sir ROBERT G. ARCHIBALD, Director of the Wellcome Tropical Research Laboratories in Khartoum, and to Dr. T. F. HEWER of the same laboratories. The sera were collected between 2nd March, 1933, and 10th October, 1934. As will be seen from the Table and Map II the south-western part of the Anglo-Egyptian Sudan contains many persons who have acquired immunity to yellow fever. The country may conveniently be divided into three regions according to the degree to which the population is immune. In the north-east the adults are without immunity to yellow fever, and we may conclude that the present population has not been exposed to infection. In the region in the south-west represented by Wau, Rumbek and Juba, even young children are found to be immune, showing that the infection has recently been active there and may now be present. To the north and south-west of this region are many towns in which only the adults are immune. The immunity in these places may have been produced by infrequent waves of infection. The results suggest that some of these towns may not have been visited in the last 18 years or more. One cannot be very positive, however, regarding the absence of immunity in the younger persons, or its significance. The testing of a larger sample of the child population might have revealed some immunes. It is the usual experience to find that the percentage of immune persons rises with age in endemic areas, and the minimum number of tests which would give dependable negative results would therefore need to be larger in the case of children than in that of adults. It is also possible that the unknown conditions of transfer of yellow fever in the Anglo-Egyptian Sudan may favour the infection of adults in places away from the towns and the escape of town-dwelling women and children, as in the "jungle yellow fever" of South America (SOPER, 1935).

PRIDIE (1934) is inclined to explain the distribution of immunity in the Anglo-Egyptian Sudan as being determined by the routes of the pilgrimages from West Africa towards Mecca. Evidence that yellow fever is now present in the Province of Bahr-el-Ghazal, where protective sera were obtained from young children, was presented by a case diagnosed as yellow fever and reported in June, 1934 (HEWER, 1934). That the infection has long been present in the Anglo-Egyptian Sudan is suggested by HEWER, who cites the case of the Sudanese soldiers recruited in Darfur and Kordofan who were sent to Mexico in 1863 and proved to be immune when exposed to yellow fever. This historical observation, and the absence of recognized severe epidemics, strongly suggests that the immunizing process in the region is of long standing, and that the area of immunity, taken as a whole, should be classed as endemic, whether the immunizing infection is intermittently introduced into the individual localities from other places or is constantly present.

A few of the results in the Table need comment. One man who gave a

SPANISH GUINEA.

In June, 1935, a shipment of one hundred blood specimens from Spanish Guinea was received. They had been collected by Dr. OTEGUI in a preliminary expedition organized by the Iglesias Amazon Expedition Advisory Council. The scientific programme had been outlined by Dr. LUIS NAJERA, Chief of the Medical Section of the Council, with the approval of Professor PITTALUGA as a member of the Council. Of ninety-nine specimens satisfactorily examined, one came from Elobey and was without protective power. The others were from Oveng, Machinda and Bimbongo. Among ninety-one sera from adult natives, nine (10 per cent.) gave protection. Eight sera from children under 16 years of age were included in the shipment. Two from Oveng and one from Machinda were without protective power. Among five from Bimbongo, one from a boy 13 years old gave complete protection and another from a boy of 8 years gave an inconclusive result (partial protection).

The evidence shows that yellow fever immunization has been taking place in Spanish Guinea in recent years. That it is now going on in the general region in which Spanish Guinea is situated is suggested by the report by BOYÉ (1934) of two probable cases of yellow fever, in May, 1934, at Port Gentil, not far south of Spanish Guinea on the coast of French Equatorial Africa.

SUMMARY AND DISCUSSION.

As the report here presented completes the general yellow fever immunity survey of Africa, it would seem appropriate at this time briefly to sum up and discuss the results of the whole investigation from its beginning in 1931 to its conclusion in 1935, including the surveys of BEEUWKES and his associates (1934) and STEFANOPOULO (BOYÉ, 1933) as well as the one we are now putting on record.

In Africa yellow fever immunity in man, as determined by blood tests in mice, is widely but irregularly distributed in a region extending from the coast of Senegal eastward for approximately 3,300 miles to the upper reaches of the White Nile in the Anglo-Egyptian Sudan. On the north this region is limited by the Sahara Desert. On the south the boundary follows the coast of the Atlantic Ocean from Senegal to the extreme northern part of Angola and then runs eastward across Angola and the southern part of the Belgian Congo. The region has a maximum width of about 1,400 miles and lies between latitudes 16 degrees North and 6 degrees South (Maps I and II).

Human blood specimens from localities scattered throughout the parts of Africa lying outside the region of immunity were found to be without power to protect mice against yellow fever virus, except in a few rare instances which have been discussed. The chance of finding an isolated individual with protective blood seems to rise with the number of sera collected and the nearness to the area of immunity. With our present knowledge we can only explain these few divergent results as probably being due (1) to infection of the blood donor with yellow fever virus when, contrary to the information given, he had visited some

distant place, (2) to sporadic infections with virus introduced into the locality or persisting there under conditions unfavourable to the spread of the infection, or (3) to an exceptional concentration of some non-specific factor in the blood.

Within the region of immunity there are areas in which no immunes were discovered, others in which only a small proportion of the adults were immune, and still others in which considerable proportions of both adults and children had protective blood. Nevertheless the region as a whole may be considered as endemic, in the sense that the disease is always present and widely distributed. To what extent it is continuously present in any particular place that shows high prevalence of immunity but has no history of yellow fever is difficult to determine, as either constant endemicity or a recent sharp epidemic might conceivably have produced the observed condition. Likewise, the places in which only a few adults are immune might either have undergone an epidemic many years earlier or have been in a constant state of low endemicity under conditions which make infective exposures infrequent. The African region of immunity may be considered as the endemic region of the Eastern Hemisphere. It is one of the two great endemic regions of the world, the other being in South America.

For convenience in discussion the African region of immunity may be divided into two parts. The western area extends to the eastern border of Nigeria and includes also the coastal region from Nigeria to Angola. The remainder of the region is the eastern area.

The western area has had numerous epidemics of yellow fever, both on the coast and in the interior, and is still having them. All the historic outbreaks of yellow fever in Africa have occurred within this area, except the two introductions of yellow fever in northern Morocco in 1804 and 1881 and the appearance of yellow fever in coastal towns of Angola up to 1899. In West Africa in the presence of the frequent urban and town epidemics in which *Aedes aegypti* is undoubtedly the vector, it would be difficult to recognize or study yellow fever perpetuated or spread in other ways than the common one previously established.

In the eastern area the situation is radically different. Yellow fever has never been recognized except for the single probable case recorded by HEWER during the course of the immunity survey. Europeans stationed in places where a large proportion of the natives are immune have never been known to contract the disease, and Europeans and natives whose blood was tested because they gave a history of a disease with symptoms suggestive of yellow fever, gave no evidence of having acquired immunity. The heavily immunized areas would therefore seem to be continuously endemic rather than epidemic, if we assume that the epidemic disease is likely to manifest its presence by some severe and characteristic cases, as it commonly does in the western area. The possibility cannot be ignored, however, that the immunizing infection may be with strains of yellow fever virus which differ from those in classic epidemics in having a lowered virulence, or a selective virulence for a different set of tissues. This is suggested by the extensive alterations brought about in the laboratory in converting the viscerotropic yellow fever virus to the neurotropic and also by minor

differences in virulence between virus strains from different sources. An epidemic with an unfamiliar strain of virus of altered virulence might be unrecognizable under present conditions, particularly among population groups possessing relatively high racial or inherited resistance, even though an occasional variant case might have resemblances to the classic disease.

Within the eastern area there is a zone of high prevalence of immunity among children and adults. The number of places sampled was not great enough to give clear boundaries for this zone or to rule out the possibility of gaps and irregular extensions. The zone lies between latitudes 3 and 8 degrees North and extends from the eastern part of the French Cameroons across French Equatorial Africa, overlapping the northern edge of the Belgian Congo, and into the Anglo-Egyptian Sudan as far as Rumbek. To the north and south of this zone there is a diminishing incidence of immunity.

The environmental conditions which favour yellow fever immunization in the zone of high prevalence of immunity in the eastern area and those which tend to confine the immunizing infection to the larger region of immunity are in great part unknown. They may be similar to those responsible for the perpetuation and limitation of the jungle yellow fever now being studied by SOPER in South America or they may be peculiar to Africa. The zone of high prevalence of immunity affords an exceptional opportunity for an intensive study by epidemiologists, pathologists, bacteriologists, entomologists and zoologists to determine (1) the symptomatology and pathology of the disease produced by the immunizing infection (2) the characteristics of the prevailing strain of yellow fever virus, (3) the identities and habits of the blood-sucking arthropod vectors, and (4) the presence or absence of warm-blooded animal hosts other than man. Persistent studies along these lines should make it possible to estimate the extent of the danger from yellow fever in Central Africa and the probability of its spread to the eastern coast. Such studies should also help to determine what precautionary measures are required.

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NIGERIAN INSECTIVORA (HEDGEHOGS AND SHREWS)—THEIR REACTION TO NEUROTROPIC YELLOW FEVER VIRUS.

BY

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FINDLAY and CLARKE (1934) have shown that the common European hedgehog is susceptible to the neurotropic yellow fever virus when inoculated intracerebrally, subcutaneously or intraperitoneally. Later, FINDLAY, HEWER and CLARKE (1935) have shown that the Sudanese hedgehog (*Atelerix albiventris*) is also susceptible to the viscerotropic strain of yellow fever virus when inoculated subcutaneously. In view of these findings it is of interest to record the results of experimental inoculations of neurotropic yellow fever virus in Nigerian hedgehogs (*Atelerix albiventris*) and shrews (*Crocidura mannii*).

HEDGEHOGS.

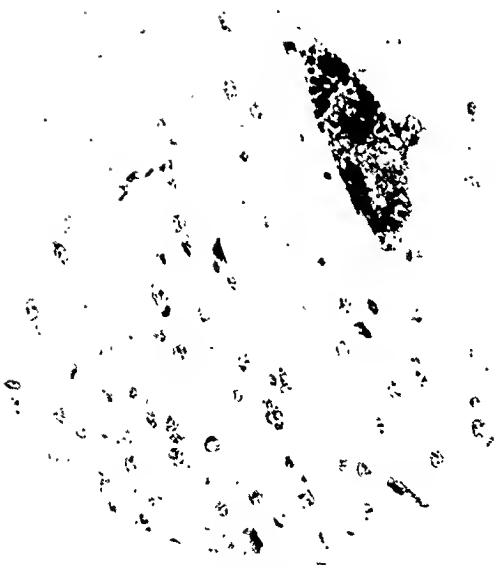
Seventeen animals were used. Thirteen were inoculated intracerebrally (including four animals used to maintain passage), with 0.15 c.c. of a 1 in 10 emulsion in normal saline of mouse brain virus fatal to mice on the 4th day. Four were inoculated subcutaneously using 1.5 c.c. of the same virus emulsion diluted 1 in 5. The latter gave entirely negative results. Of the thirteen inoculated intracerebrally, seven died (or were killed *in extremis*) at periods varying from 9 to 13 days, average 10.5 days, from the date of inoculation. All the animals showed paralytic symptoms and were unable to coil up when touched or alarmed: they lay in an extended position, were unable to move and became rapidly moribund within 24 to 48 hours after the development of symptoms. In one instance the virus was passaged through four hedgehogs and was finally transferred to mice (intracerebral inoculation), both unfiltered and after passage through an unused Berkefeld V. filter. The six mice used in

* The author is indebted to the Director, Medical and Sanitary Services, Nigeria, for permission to publish, to Mr. BEATON, Veterinary Department, Kano, for his kindness in obtaining a supply of hedgehogs and to the Senior Health Officer, Lagos, for his help in procuring the shrews.

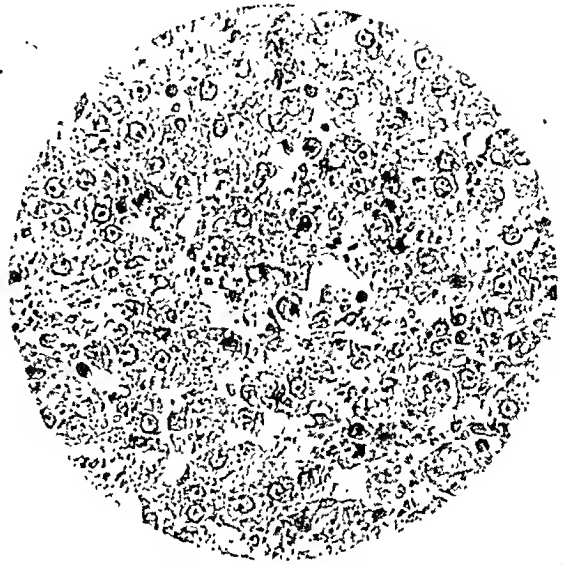
10

HISTOPATHOLOGY OF YELLOW FEVER IN THE HEDGEHOG.

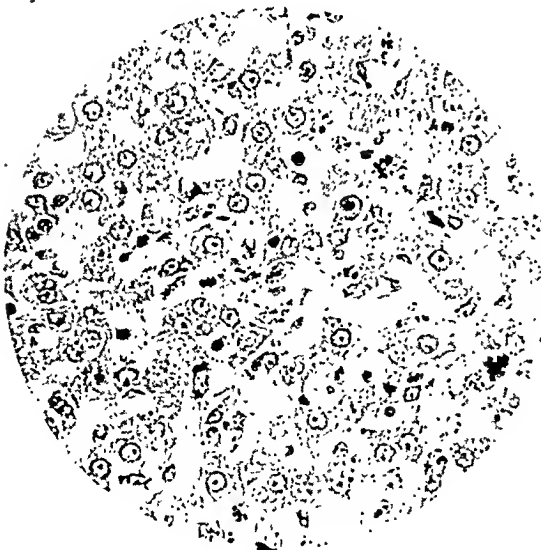
(*Haematoxylin and eosin staining.*)



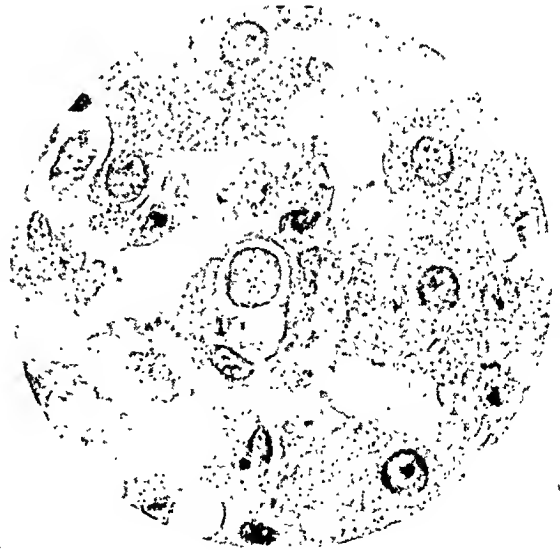
1. Cerebral cortex (hedgehog, first passage).
Perivascular infiltration. $\times 375$.



2. Liver (hedgehog, third passage). Dis-
organisation and necrosis of the hepatic
cells. $\times 375$.



3. Liver (hedgehog, third passage). Infil-
tration of the tissue with leucocytes,
necrosis and pigmentation of K  pffer cells.
 $\times 375$.



4. Liver (hedgehog, fourth passage). Well
marked "Councilman" lesion and hyper-
chromatic nuclei. $\times 1000$.

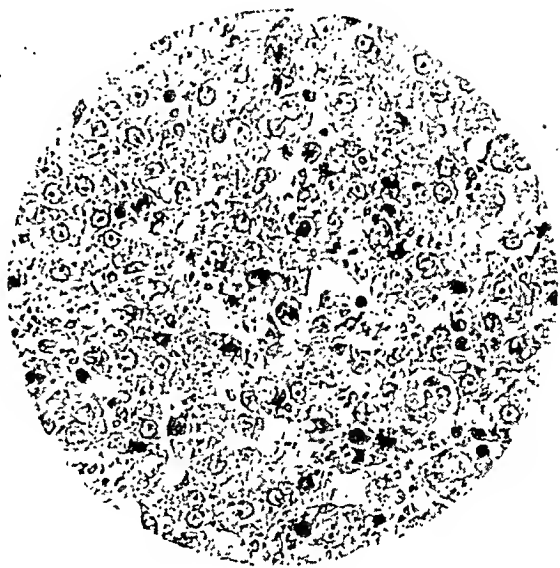
Microphotographs by Mr. J. E. Knight.

HISTOPATHOLOGY OF YELLOW FEVER IN THE HEDGEHOG.

(Haematoxylin and eosin staining.)



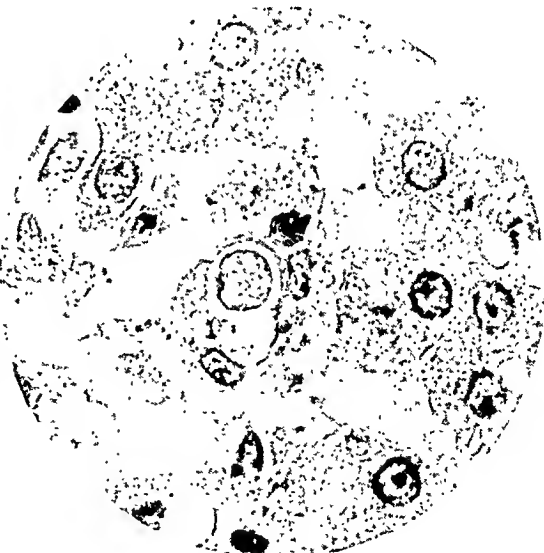
1. Cerebral cortex (hedgehog, first passage). Perivascular infiltration. $\times 375$.



2. Liver (hedgehog, third passage). Disorganisation and necrosis of the hepatic cells. $\times 375$.



3. Liver (hedgehog, third passage). Infiltration of the tissue with leucocytes, necrosis and pigmentation of Kupffer cells. $\times 375$.



4. Liver (hedgehog, fourth passage). Well marked "Councilman" lesion and hyperchromatic nuclei. $\times 1000$.

Microphotographs by Mr. J. E. Knight.

the first experiment died on the 5th day and those used in the second experiment died on the 5th or 6th day. Cultures of the hedgehog brain virus emulsions were made on agar and in broth after each passage with negative results.

Pathological Appearances.

Macroscopic.

Brain.—Slight congestion of the meningeal vessels was noted.

Liver.—Faint mottling was seen in one of the passage animals (third passage). The remainder showed congestion.

Stomach.—No changes were seen in this organ in any of the animals examined.

Microscopic.

Paraffin sections were prepared and in addition, frozen sections were made of the liver and stained for fat (sudan III).

Brain.—Congestion of the vessels was present in all those examined together with perivascular infiltration with lymphocytes. The meninges, also, were congested and oedematous and were scantily infiltrated by leucocytes of the mononuclear type.

Liver.—Fat staining revealed the presence of scattered groups of degenerated liver cells. These cell groups did not seem to have any definite zonal distribution. In the paraffin sections there was congestion of the vessels, pigmentation of the Küpffer cells, jaundice and diffuse infiltration of the tissue with lymphocytes and occasional polymorphs, the infiltration becoming condensed in the region of Glisson's capsule.

Necrotic changes were definitely present in two of the livers examined (those of the third and fourth passages). These showed patchy disorganisation of the hepatic tissue wherein the cells were undergoing a form of granular necrosis with the formation of "Councilman bodies." The nucleus of many of the hepatic cells showed hyperchromatic changes and contained what appeared to be a finely granular acidophilic substance, more readily appreciated when Giemsa stain was used.

Stomach.—Slight vascular congestion only was observed.

SHREWS.

Twelve shrews (*Crocidura mamm*) were inoculated with neurotropic yellow fever virus. Eight animals received 0.1 c.c. of a 1 in 10 mouse brain emulsion in saline intracerebrally and four received 0.5 c.c. of a 1 in 5 emulsion subcutaneously. None of the animals developed symptoms. A protection test, made with the pooled sera (blood obtained by cardiac puncture), of four normal shrews, was negative.

COMMENT.

The results obtained indicate that the local variety of hedgehog (*Atelerix albiventris*) is susceptible to the neurotropic form of yellow fever virus when inoculated intracerebrally but not when inoculated subcutaneously. The findings in two of the livers examined indicate neurotropic changes in this organ. As no viscerotropic yellow fever virus was available it was not possible to determine the susceptibility of the local hedgehog thereto. Should it be subsequently demonstrated that the animals are susceptible to the viscerotropic virus it is suggested that they might be used for diagnostic purposes in lieu of the costly *Macacus rhesus* monkey. The maintenance of a stock of hedgehogs would entail some initial difficulty in obtaining healthy animals as they do not travel well being liable to develop septiceamic sores, particularly on the limbs. Healthy animals have been maintained in Lagos for several months. They were kept in separate cages and given a varied diet of chopped fresh liver, raw meat and ground nuts together with a plentiful supply of water. Bread and milk was given for a time but was partaken of but sparingly.

CONCLUSION.

The hedgehog common in Northern Nigeria is susceptible to the neurotropic strain of yellow fever virus when inoculated intracerebrally.

Shrews obtained in Southern Nigeria have not been found to be susceptible to the same virus.

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THE SUSCEPTIBILITY OF NIGERIAN HEDGEHOGS TO YELLOW FEVER.

BY

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AND

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In view of the susceptibility of hedgehogs (*Atelerix albiventris*) from the Sudan to the viscerotropic yellow fever virus (FINDLAY, HEWER and CLARKE, 1935) and the results obtained by E. C. SMITH (1936) with Nigerian hedgehogs infected with neurotropic yellow fever virus it appeared to be of interest to test the susceptibility of Nigerian hedgehogs to the viscerotropic strain of yellow fever virus.

Six hedgehogs obtained from the neighbourhood of Kano, Northern Nigeria, were despatched to this country by Dr. E. C. SMITH to whom our thanks are due. On arrival, they were tested for their susceptibility to French and Asibi yellow fever virus (viscerotropic strains). Two hedgehogs were inoculated intraperitoneally, one intracerebrally and two both intracerebrally and intraperitoneally, European hedgehogs being inoculated at the same time as controls. The European hedgehogs all died from yellow fever in from 5 to 8 days, but none of the Nigerian hedgehogs succumbed or showed any definite illness.

In order to determine whether this resistance was due to immunity as the result of previous infection occurring in Northern Nigeria, the sixth hedgehog was killed and its blood tested for immunity to yellow fever. No immune bodies were found in the blood although two of the Nigerian hedgehogs that had been inoculated intraperitoneally with yellow fever a month previously did show immune bodies in the blood.

* Working with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

In association with SMITH's experiments it must therefore be concluded that the Nigerian hedgehogs employed were not immune to yellow fever as a result of previous infection, but possessed a racial resistance.

This resistance is all the more surprising as the hedgehogs from Kano, Northern Nigeria are very similar to, if not identical with, those from Omdurman, Anglo-Egyptian Sudan. Of these Sudanese hedgehogs, three out of four died with necrosis of the liver as a result of intraperitoneal injection of viscerotropic yellow fever virus.

Some uncertainty appears to exist as to whether the Northern Nigerian hedgehog differs from that found in the Sudan. THOMAS and WROUGHTON (1907) described the Northern Nigerian hedgehog as *Atelex spiculus*, the type species from Maifoni near Lake Chad being now in the British Museum (Natural History), South Kensington. Further investigation, however, throws doubt on the validity of *A. spiculus* which must be regarded as probably identical with *A. albiventris* (= *Erinaceus pruneri*). The investigations here described suggest that there may be a physiological difference between the Sudanese and Nigerian strains. In this connection it is interesting to note that the individuals highly susceptible to yellow fever were obtained from Omdurman, Anglo-Egyptian Sudan, where there has never been recorded an epidemic of yellow fever and the disease is certainly not endemic. The resistant individuals, on the other hand, were obtained from Kano, Northern Nigeria, where epidemics of yellow fever are by no means rare and the disease is possibly endemic.

CONCLUSION.

1. Hedgehogs, probably *Atelex albiventris*, obtained from Kano, Northern Nigeria, were found to be highly resistant to infection with viscerotropic yellow fever virus.

2. This resistance was not due to the presence of immune bodies in the blood prior to the experimental inoculation.

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YELLOW FEVER IMMUNE BODIES IN THE BLOOD OF AFRICAN ANIMALS.

PRELIMINARY OBSERVATIONS.

BY

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With the demonstration by REED, CARROLL and AGRAMONTE (1901) that yellow fever is transmitted by *Aedes aegypti* the main factors in the epidemiology of the disease appeared to be once and for all established: in the absence of either susceptible human beings or mosquito vectors the disease would cease. The eradication of mosquitoes from Havana and the subsequent disappearance of yellow fever from the whole of the West Indies was thought to establish the fact that the elimination of mosquitoes from large urban key centres would automatically lead to the elimination of yellow fever from the surrounding country. After a few years, however, it was recognized that such a sequence of events does not necessarily follow. No satisfactory explanation, however, was forthcoming till in 1928 BAUER, following on the suggestion of MANSON (1914), showed that *Aedes aegypti* was not the only member of the Culicidae that could play the part of an insect vector of yellow fever. In a short time a number of other species of mosquito both in Africa and South America were found capable of transmitting the virus of yellow fever by their bites; later, in South America, actual outbreaks of yellow fever were recorded in the absence of *Aedes aegypti* in a number of rural areas such as Chanaan, Espirito Santo, Brazil; San Ramón, Bolivia; and Coronel Ponce, Matto Grosso (SOPER, 1935).

* Working with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

The further possibility that in nature certain animals may act as alternative hosts for the virus of yellow fever has already been suggested. Thus BALFOUR (1915) described a heavy mortality in the red howler monkey (*Alouatta seniculus*) in association with an outbreak of yellow fever in Trinidad. He also stated that in Gibraltar during the epidemic of 1828 the apes on the Rock succumbed in large numbers: the Barbary ape (*Macaca sylvanus*) has been shown by PETTIT and STEFANOPOULO (1930) to die from yellow fever. Unfortunately, BALFOUR failed to state the source from which his information was derived while contemporary accounts of the Gibraltar epidemic consulted by us fail to mention any mortality among monkeys.

Under laboratory conditions many species of African animals, including monkeys, can be infected with yellow fever although they do not die or show any clinical symptoms of ill health following injection of the virus. The virus, however, circulates for some days in the peripheral blood stream while later immune bodies are developed. Normal wild animals if bitten by mosquitoes infected with yellow fever virus may therefore act as alternative hosts to man and if again bitten during the period when the virus is present in the blood stream they may serve to maintain the supply of infected mosquitoes. DAVIS and PHILIP (1931) have pointed out that *Aedes aegypti* and other potential yellow fever vectors by no means feed exclusively on man.

During the past two years, therefore, an attempt has been made to examine the bloods of wild and domestic animals from Africa for the presence of immune bodies to yellow fever. While these experiments were in progress SOPER (1935*a* and *b*) recorded the fact that immune bodies to yellow fever had been found in the bloods of certain South American monkeys obtained from rural areas in South America. The positive sera are found among at least four different species, *Callicebus ornatus*, *Saimiri sciurea*, *Cebus apella* and *Hapale* sp. (SOPER, 1935*b*).

APE AND MONKEY BLOODS.

Bloods of apes and monkeys were obtained during 1934 from the Gold Coast and from French Guinea: during 1935 from Bathurst, Gambia and from the Belgian Congo.*

The primates examined comprised six chimpanzees and nineteen other monkeys. As controls, seventeen Asiatic monkeys were examined—eleven rhesus monkeys (*Macaca mulatta*) and six crab-eating macaques (*Macaca irus*).

The usual intraperitoneal mouse protection test was employed. Of the control monkeys, all were negative: of the African monkeys, three were positive, a chimpanzee from French Guinea, a baboon from the Belgian Congo and a

* For assistance in obtaining bloods from the Gambia we are greatly indebted to Dr. H. J. BIRMINGHAM and Mr. ERIK JONSON, while our sincere thanks are also due to Dr. P. BRUTSAERT, Léopoldville, for sending us bloods from the Belgian Congo, and to Miss FANNY WALDREN who collected the bloods in the Gold Coast Colony.

Colobus monkey from the Gold Coast. The results are shown in Table I. The positive sera were tested at least twice to ensure that the first test was not due to adventitious factors and all three were found to protect either in a dilution of 1 in 4 or 1 in 8.

Shortly before the monkey bloods were obtained from the Gambia an

TABLE I.

BLOODS OF AFRICAN APES AND MONKEYS TESTED FOR IMMUNITY TO YELLOW FEVER.

No. of Monkey.	Species.	Locality.	Result.
1	<i>Anthropopithecus troglodytes</i> Linn. Chimpanzee	French Guinea	P
2	" " " "	" "	NP
3	" " " "	" "	NP
4	" " " "	" "	NP
5	" " " "	" "	NP
6	" " " "	" "	NP
7	<i>Papio</i> sp. Baboon	Belgian Congo	P
8	<i>Mandrillus sphinx</i> Linn. Mandrill	Gambia	NP
9	<i>Colobus badius</i> (sub. sp. nov.)	Goaso, Gold Coast	P
10	<i>Erthrocebus patas</i> Schreber. Patas monkey	Mampong, Gold Coast	NP
11	<i>Allenopithecus nigroviridis</i> Pocock. Dwarf green monkey	Belgian Congo	NP
12	<i>Cercopithecus diana roloway</i> Schreber. Gold Coast Diana monkey	Goaso, Gold Coast	NP
13	<i>C. mona lowei</i> Thomas. Lowe's Mona monkey	" "	NP
14	<i>C. eephus</i> Linn. Moustached guenon	Belgian Congo	NP
15	" " " "	" "	NP
16	<i>C. neglectus</i> Schlegel. Schlegel's guenon	" "	NP
17	<i>C. aethiops sabaes</i> Linn. Green monkey	Bathurst, Gambia	NP
18	" " " "	" "	NP
19	" " " "	" "	NP
20	" " " "	" "	NP
21	<i>C. aethiops cynosuroides</i> Scopoli. Malbrouck guenon	Belgian Congo	NP
22	<i>C. galeritus agilis</i> E. Rivièrè. Agile mangabey	" "	NP
23	" " " "	" "	NP
24	" " " "	" "	NP
25	" " " "	" "	NP

P = Protection.

NP = No protection.

outbreak of yellow fever had occurred in the town of Bathurst. This epidemic was of the typical urban type and there is no evidence that it spread to the surrounding country districts.

In French Guinea at the time that the bloods were obtained from chimpanzees, cases of yellow fever were occurring. At Goaso, Gold Coast, on the other hand, there had been no history of any recent outbreak. Through the

kindness of Dr. DAVID DUFF, Director of Medical and Sanitary Services, Gold Coast, Dr. R. D. REID, of the Medical Research Laboratory, Accra, was enabled to obtain the bloods of twelve children from Goaso under 12 years of age, all of whom were born and had spent their whole lives in that neighbourhood. One of these children protected.

Although, in the case of the Belgian Congo, the exact locality from which the positive baboon came is unknown, it is believed to be from the northern

TABLE II.

BLOODS OF MAMMALS (OTHER THAN PRIMATES) AND BIRDS FROM AFRICA EXAMINED FOR IMMUNE BODIES TO YELLOW FEVER.

No.	Species.	Locality.	Result.
1	<i>Epomophorus</i> sp. Fruit-eating bat	Bathurst, Gambia	NP
2	" "	" "	NP
3	" "	" "	NP
4	" "	" "	NP
5	<i>Ovis ares</i> Linn. Sheep	" "	NP
6	" " "	" "	P
7	" " "	" "	NP
8	" " "	" "	P
9	" " "	" "	NP
10	" " "	" "	NP
11	" " "	" "	NP
12	" " "	" "	NP
13	<i>Bos taurus</i> Linn. Ox	" "	NP
14	" " "	" "	NP
15	" " "	" "	NP
16	<i>Euxerus erythropus</i> Geoff. Ground squirrel	Wa, Gold Coast	NP
17	<i>Arvicanthus rufinus</i> Temminck	Pong Tamale, Gold Coast	NP
18	<i>Taterona guineae</i> sub. sp. nov.*	" "	NP
19	<i>Lemniscomys barbarus nigeriae</i> Thos.	" "	NP
20	<i>Cricetomys gambianus</i> Waterhouse. Giant rat	" "	NP
21	<i>Gallus</i> sp. Hen (1)	Bathurst, Gambia	NP
22	" " (6)	" "	NP
23	" " (5)	" "	NP
24	" " (2)	" "	NP
25	<i>Necrosyrtes monachus monachus</i> Sclater. Common vulture	" "	NP

P = Protection. NP = No protection. **Proc. Zool. Soc.* (MS.).

half of the country where varying numbers of positive sera are found among human beings.

OTHER ANIMAL BLOODS.

In Table II are shown the results obtained with bloods from a certain number of wild and domestic animals and birds. All the bloods were negative

except those of two sheep from the Gambia. One of these sheep protected in a dilution of 1 in 64, the other in 1 in 8.

As a control, the bloods of six English sheep were obtained through the kindness of Dr. R. A. O'BRIEN, Director of the Wellcome Physiological Research Laboratories, Beckenham. These sheep had never been out of England but the blood of one of them protected in a dilution of 1 in 2 though not in 1 in 4. As this sheep could never have been exposed to yellow fever infection it is possible that false positive protection tests may occasionally be given by the blood of certain Ovidae. It is, however, of interest to note that DAVIS and PHILIP (1931) found that *Mansonia africana* and *Culex thalassius*, potential yellow fever vectors, both fed under natural conditions on sheep and goats.

DISCUSSION.

When STOKES, BAUER and HUDSON (1928) first showed that Asiatic, but not African, monkeys succumbed to yellow fever it was suggested that the latter were immune to yellow fever. Later, however, it was found that African monkeys were merely resistant to the virus and not in reality immune. The preliminary observations here described suggest that occasionally true immunity may be found in African apes and monkeys. If these observations are confirmed and extended they suggest that in rural as opposed to urban areas monkeys may play some part in maintaining the continued existence of yellow fever. As in South America (SOPER, 1935c) so in Africa a distinction will then have to be made between the epidemiology of urban and rural yellow fever.

Further work, however, is obviously required to determine whether the mouse protection test for immune bodies to yellow fever is as specific in the case of animal bloods as it is in the case of human bloods, where no serious criticism of the specificity of the test has yet been advanced. In this connection it may be said that the bloods of rhesus monkeys infected with malaria (*Plasmodium knowlesi*) do not give a positive protection test for immune bodies to yellow fever, nor does the serum of animals immune to equine encephalomyelitis,* a virus disease of Equidae also transmitted by *Aedes* mosquitoes and widely distributed in both North and South America.

It may, however, be interesting to note that recently RAMON and ERBER (1935) have found that 53 per cent. of African monkeys show significant amounts of diphtheria antitoxin in their bloods and, although diphtheria is always regarded as being extremely rare in Africa, it has been possible in certain cases to isolate diphtheria bacilli from their throats.

CONCLUSIONS.

1. Of twenty-five normal chimpanzees and monkeys from Africa, whose bloods were examined for the presence of immune bodies to yellow fever, three

* For this serum we are greatly indebted to Dr. E. WESTON HURST of the Lister Institute.

were positive, a chimpanzee from French Guinea, a *Colobus* monkey from the Gold Coast and a baboon from the Belgian Congo. The bloods of seventeen Asiatic monkeys were negative.

2. Human sera protecting against yellow fever have been obtained from the same areas.

3. The bloods of two sheep from the Gambia protected against yellow fever but one of six English sheep used as a control also protected though only in a very low dilution.

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OBITUARY.

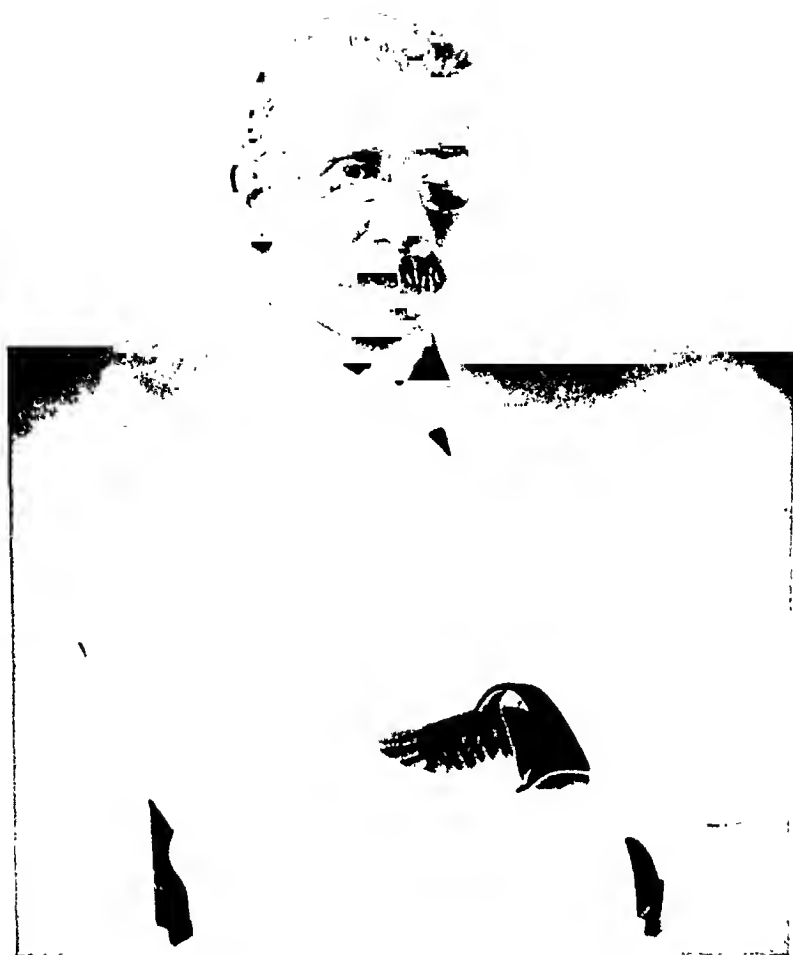
ETTORE MARCHIAFAVA.

1847-1935.

On 24th October, Professor MARCHIAFAVA died serenely in Rome in his 88th year. Physicians of great wisdom, high integrity and warm humanity have the special reward of being beloved as well as admired. MARCHIAFAVA was the personal physician of three Popes and of the Royal House of Savoy, but he was particularly the physician of the poor people of Rome. He was active and untiring even in his extreme old age in every move to improve medical assistance and promote measures for the prevention of disease, so that at his simple funeral service the church was unable to hold the people of every class—Senators, members of the Government, doctors, scientists, labourers and sisters of charity—who came to pay tribute to a great citizen as well as a great scientist. He had been made Senator of Italy in 1913.

To the throng of his old students he was both an investigative pathologist and a friendly, enthusiastic teacher, striving always to link the anatomical with the clinical picture of disease. He became full Professor in the University of Rome at 36, retiring in 1922 at the age of 75. He wrote and spoke with charm and clarity, and possessed a wide humanistic culture, being especially at home in Latin and Greek.

To the world, of course, he was one of the foremost malariologists, a member of that famous Italian group which included GOLGI, CELLI, GRASSI, BIGNAMI and BASTIANELLI, and which at the turn of the last century, developed the fundamental discoveries of LAVERAN and ROSS, building the structure of our present knowledge of the *Plasmodium* species and their transmission by a particular genus of mosquito. To MARCHIAFAVA belongs the honour of describing after patient research the forms and life cycle of *P. falciparum*, noting first the pigment in 1879 before the parasite itself was discovered, identifying the ring forms in 1883 and finally (1889) correlating the whole developmental cycle of the parasite with the course of estivo-autumnal fever, just as GOLGI had done with *P. vivax* and *P. malariae*. In 1902, with BIGNAMI he published his great book *La Infezione Malarica*. This was a fundamental work which no malariologist to-day can afford to neglect. As a research worker and as a clinician MARCHIAFAVA was sternly methodical and exact, and hence this accurate record of his observations has not been rendered obsolete by the advance of knowledge, but to use ST. BEUVE's definition of a classic, remains "easily contemporaneous with all time." The jealousies and bitter polemics which characterized, for some



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curious reason, the early history of malaria discovery, left MARCHIAFAVA untouched. There was an unmistakable modesty, integrity and highmindedness about him that in the evening of his life provided him with a multitude of friends and no enemies.



He was elected to many Italian and foreign scientific groups in recognition of his scientific attainments, and was an Honorary Fellow of the Royal Society of Tropical Medicine and Hygiene from its foundation in 1907. He went to London in 1926 to receive the Manson Medal from this Society. He was active mentally and physically to the end, and a frequent visitor to the library of the Malaria Experiment Station in Rome, helpful and unassuming always as becomes a great man.

L. W. HACKETT.

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

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on Thursday, 16th January, 1936.

Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*,
in the Chair.

PAPER.

SOME POINTS IN THE EPIDEMIOLOGY OF MALARIA ARISING OUT OF THE STUDY OF THE MALARIA EPIDEMIC IN CEYLON IN 1934-35.

BY

COLONEL C. A. GILL, M.R.C.P. (LONDON), D.P.H., D.T.M. & H., I.M.S. (RETD.)*

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6. CONCLUSIONS.

*I should like to take this opportunity of acknowledging my great indebtedness to Dr. R. BRIERCLIFFE, *C.M.G.*, Director, Medical and Sanitary Services, Ceylon, for the assistance freely and generously given me both during my stay in Ceylon and since my return to England.

I must also thank Professor W. W. JAMESON for his kindness in placing a room and the library of the London School of Hygiene and Tropical Medicine at my disposal, and, finally, I must express my gratitude to Dr. C. M. WENYON, Director, Wellcome Research Institution, for kindly arranging for the preparation of the lantern slides.

1.—INTRODUCTORY.

When I was first honoured with an invitation to read a paper on the malaria epidemic in Ceylon my intention was to give a full and complete account of the epidemic, but when I came to prepare the paper I found it would take so long to deliver that it would leave no time for discussion. I therefore decided to confine my remarks to the epidemiological aspect of the epidemic, and more especially to the mechanism of the epidemic wave. I shall therefore make no reference to the practical measures taken to cope with the epidemic, and I shall only refer to its disastrous effects in order to emphasize the powerlessness of modern medical science to prevent the outbreak of malaria epidemics or to check materially their course.

The toll of life exacted by the Ceylon epidemic was approximately 100,000, whilst the sufferers probably numbered between one-third and one-half of the total population of the island. I will not attempt to appraise these losses in terms of money, but the cost to the State of relief measures was approximately £350,000. The epidemic may perhaps be regarded as an exceptional horror, due to causes beyond human control, but can it be contended that the control of endemic malaria in the tropics is in much better case?

It is true that in towns and cities, and in small "closed" populations and communities, the control of malaria does not usually present insuperable financial or administrative difficulties, but what of the large free population of rural areas, numbering more than 80 per cent. of the total? In Ceylon, for example, it was not possible to suggest any practicable scheme for controlling malaria in an area embracing nearly three-quarters of the island, most of which is undeveloped and almost unpopulated solely on account of malaria. In so far as this and other similar areas in the tropics are concerned, it may be said that, despite the great advance of knowledge during the past 50 years, the control of malaria, as a practicable proposition, is almost as far to seek as it was a century ago.

The object of these remarks is not to depreciate what has been achieved in the past or is being attempted in the present, but to emphasize the view, which I believe is widely held, that there is little hope, within any time that can be foreseen, of making substantial progress in combating the greatest scourge of the tropics until new and more effective methods of prevention and treatment become available. And if this is so, it is imperatively necessary to fill some of the gaps in our knowledge of the endemology and epidemiology of malaria, in the hope that by obtaining a full and complete knowledge of the life-cycle of the malaria parasite, which of course covers a wider field than the life-history of the malaria-carrying mosquito, it will be possible to broaden the basis of anti-malaria measures and to increase their effectiveness.

The malaria epidemic in Ceylon provided an opportunity of studying malaria under extremely favourable conditions, and my object this evening is to place before you certain observations which, if correctly made and rightly

interpreted, appear to throw light upon certain obscure aspects of the endemiology and epidemiology of malaria and help to explain the cause of the recent epidemic.

2.—SUMMARY OF PREVIOUS WORK ON EPIDEMIC MALARIA.

In most, if not in all, paludic countries malaria occasionally assumes abnormal prevalence, and when its incidence is unusually high over wide areas, a generalized epidemic is said to occur, and when the epidemic is sufficiently extensive, malaria is said to prevail in pandemic form. These epidemics may exhibit every grade of intensity from a slight exaggeration of the normal seasonal wave of morbidity and mortality to a fulminant epidemic associated with almost universal sickness and a death-rate many times the normal. Fulminant epidemics occur rarely, but the epidemics that decimated Mauritius in the year 1867, the Punjab in 1908, and Ceylon in the year 1935 are outstanding examples of virulent regional epidemics.

Minor epidemics, although they occur more frequently, often escape recognition or are merely regarded as "bad fever years," and it thus comes about that almost all that is known in regard to the natural history of epidemic malaria is derived from the study of major epidemics; and it is no doubt largely because major epidemics occur more frequently in Northern India than elsewhere that existing knowledge regarding their causation is mainly due to investigations conducted in India. CHRISTOPHERS (1911) was the first to study a great regional epidemic of malaria, and, in his classical report on the epidemic in the Punjab in the year 1908, he not only established all the salient features of this epidemic, but he foreshadowed the lines and suggested the methods of future research, and, by so doing, placed all subsequent workers in the same field, and more especially the writer, under a great and abiding obligation.

CHRISTOPHERS showed that regional epidemics had occurred periodically in the Punjab ever since vital statistics were recorded, and he noted that their onset was characterized by the sudden and almost simultaneous outbreak of sickness over wide areas followed by high mortality mainly amongst infants and young children. He showed that in the Punjab excessive rainfall and flooding was the important determining cause of these epidemics, and that scarcity was an almost equally powerful influencing factor. He stressed the peculiar focal character of the epidemics, their intensity being greatest in the centre and fading towards the periphery, "vividly calling to mind such phenomena as areas of low and high barometric pressure." As regards anopheline prevalence, he remarked that the close association of the epidemic with flooding suggested something more than the provision of unusual facilities for the reproduction of the species in pools.

He concluded that "the exact mechanism of epidemic causation is still unknown, but the epidemic condition appears to be due to an excessive seasonal increase of the parasite-rate, fluctuations in which occur even in healthy years." Finally, he stressed the importance of the quantitative study of malarial

infections, and he showed, by experiments with avian malaria, that the severity of infection depended, not so much upon the number of infected mosquitoes, as upon the number of sporozoites injected at each bite, and that this, in turn, depends upon the number of gametocytes in the peripheral blood of the avian host at the time it was bitten.

The investigation was continued by GILL (1921) whose experimental work on the influence of atmospheric humidity upon the life-history of mosquitoes and their power to transmit infection led him to conclude that high atmospheric humidity, in association with high temperature, exercised a profound effect upon the epidemiology of malaria by favouring the numerical prevalence, longevity, and metabolic activity of the insect-carrier, and by accelerating the sexual cycle of the malaria parasite in the insect-host. He also made the pertinent, although negative, observation that relative humidity does not exercise any influence upon the number or rate of development of oöcysts. He concluded therefore that excessive rainfall and flooding favoured the occurrence of malaria epidemics mainly by their effect in creating and maintaining an environment highly favourable to the bionomics of the insect-carrier and to its power to transmit infection, but he also suggested (GILL, 1928) that the sudden rise of atmospheric humidity during the pre-epidemic period might possibly exercise a direct effect upon the malaria parasite in the tissues of the human host.

The experimental work was repeated by MAYNE (1930) who found that *Anopheles culicifacies* was more sensitive to atmospheric humidity than are certain other species, whilst COVELL (1928), MAYNE (1928) and others have confirmed the observation of BENTLEY (1911) that the optimum conditions for mosquito transmission occurred when, in association with high atmospheric temperature, the mean relative humidity was in the vicinity of 80 per cent.

GILL (1928), as the result of the study of an epidemic in the year 1921 from before its onset until after its termination, showed that the epidemic started suddenly at a time when the spleen-rate and the parasite-rate were extremely low, that the epidemic wave was associated with a sudden increase in the average intensity of infections (infestation index) and was followed by a rise, at a slower rate, of the parasite-rate and the spleen-rate, whilst the mortality commenced to rise about four weeks after the onset of sickness. He showed that both the common species of malaria parasites were concerned in the production of the epidemic wave, *P. vivax* greatly predominating during the early stages of the epidemic, and *P. falciparum* towards its close. Finally, the epidemic was associated with a great numerical increase of *A. culicifacies* and a rise in the infection-rate of this species.

COVELL and BAILY (1932), as the result of the study of a malaria epidemic in Northern Sind in the year 1929, confirmed the substantial accuracy of the above observations, and agreed with GILL that an epidemic of malaria was essentially caused by a sudden increase of the infection quantum at a time when communal immunity is absolutely and relatively low. In regard to the mechanism

of epidemic causation GILL (1928) stated " that it is not permissible to conclude that an epidemic is *solely* associated with the occurrence of a quantitative change in these factors " but he held " that amongst the concurrent, concomitant, and consequential phenomena associated with an epidemic of malaria changes of a quantitative order constitute a conspicuous feature." (The italics are in the original.)

This cautious attitude towards definitive conclusions was enjoined by three special considerations. In the first place no adequate explanation was forthcoming of the occurrence of a sudden and widespread outbreak of sickness at a time when the parasite-rate was extremely low (5 per cent.) and when gametocytes could scarcely be detected by ordinary methods of examination in the peripheral blood. In this connection GILL remarked (1928) " if it could be shown that some atmospheric state was capable of inducing a sudden increase in the number of sexual parasites in the peripheral blood of human carriers during the pre-epidemic period, all difficulties would disappear, but so far it has not been possible to verify the accuracy of this surmise." Secondly, in view of the essential part played by excessive monsoon rainfall in determining epidemics of malaria in the Punjab, it was necessary to account for the fact that in Bombay, Madras, Bengal, and Assam an even larger excess of monsoon rainfall does not apparently favour the occurrence of an epidemic, and to explain the still more puzzling fact that in many parts of these provinces (and also in certain other tropical countries) epidemics of malaria were reported to occur in association with deficient monsoon rainfall. Finally, in view of the important influence attributed to an unstable climate in determining long periods during which the transmission of infection is in abeyance followed by short periods during which the environment is highly favourable to transmission, it was necessary to account for the fact that the greatest malaria epidemic on record occurred in a small tropical island (Mauritius) where the temperature and humidity conditions are normally favourable to transmission throughout the year.

Pending investigations which would permit of the removal of these difficulties it was clear that only tentative conclusions were permissible, and the verdict of SCHÜFFNER (1931) that the cause of these epidemics was still obscure was fully justified ; but great epidemics of malaria are of rare occurrence, more especially in the tropics, and until the outbreak of the recent epidemic in Ceylon no opportunity has presented itself of investigating a major epidemic of malaria in the tropics.

3.—THE MALARIA EPIDEMIC IN CEYLON.

(A) THE TERRAIN.

Topography and Physiography.

The island of Ceylon, which has an area of 25,332 square miles, is situated to the south of the Indian peninsula between 5° 5' and 9° 50' north latitude.

It is pear-shaped ; its greatest length is 270 miles and its greatest breadth is about half its maximum length. (Fig. 1.)

The main topographical feature is the central mountain system with peaks rising to between 7,000 and 8,000 feet above sea-level. Surrounding the central massif is the sub-montane tract at an elevation of 1,000 to 2,000 feet, with higher peaks, representing outliers of the main system, rising to 3,000 feet. Between the sub-montane tract and the sea is an undulating plain, thickly clothed with forest and jungle, which slopes gradually to the sea.

The island is well watered, but the rivers, which radiate in all directions from the central massif to the sea, are nearly all small and become dry during rainless periods, except near their mouths.

The longest river is the Mahaweli Ganga, which, rising near Kandy on the west side of the mountain range, flows north-east to enter the sea at Trincomalee. A special importance attaches to this river and to the four rivers flowing due west from the mountain tract to the sea, owing to the fact that they were thought to have played a fundamental part in the causation of the epidemic. These rivers, from north to south, are the Deduru Oya, Maha Oya, Kelani Ganga, and Kalu Ganga, which enter the sea at Chilaw, Negombo, Colombo, and Kalutara, respectively. These rivers usually contain water throughout the greater part of their course, but they also become dry during a severe drought, when innumerable shallow sheets of water with sandy bottoms and rocky pools, which are favourite breeding places of *A. culicifacies*, are left in their exposed beds.

Climate.

The climate of Ceylon, as would be expected of a small tropical island situated less than 10° from the equator, is extremely equable as well as hot. Excluding the mountain tract, which mostly lies outside the malaria zone, the mean monthly temperature is of the order of 80° F. The diurnal range rarely exceeds 15° F. and the minimum temperature does not often fall below 65° F. So far as the low country is concerned, the atmospheric temperature is therefore favourable almost everywhere and at all times of the year to the metabolic activity of anophelines and to the completion of the exogenous cycle of the malaria parasite.

The mean monthly relative humidity in the plains is characterized by its relative constancy at a high level throughout the year.

In the extreme south-west (Galle) the mean monthly humidity (as measured at 9.30 a.m. and 3.30 p.m.) ranges between a minimum of 74 per cent. in April and a maximum of 84 per cent. during the south-west monsoon (June to September), whilst in Colombo and other districts along the west coast the relative humidity ranges between 68 and 78 per cent. In the districts bordering on the east coast and in the south-east of the island the range is greater, the mean relative humidity at Trincomalee varying between 59 per cent. in July and 79 per cent. in December, whilst in the central districts, which include

those severely affected by the epidemic, the mean monthly relative humidity varies between 60 and 70 per cent. Owing to their proximity to the sea, the severe drought during the months of July to September, 1934, exercised little effect upon atmospheric humidity in the coastal districts, the relative humidity in September, at the height of the drought, being only 1 per cent. below normal in Colombo, Jaffna, and Trincomalee, and 2 per cent. in Galle, but in the central districts the drop in relative humidity was greater, being, in September, 13 per cent. in Kurunegala, 10 per cent. in Kandy, 7 per cent. in Anuradhapura and 4 per cent. in Ratnapura.

The most remarkable feature of the climate of Ceylon is the variable distribution of the rainfall in time and space, which is mainly due to the influence of the central mountain system upon the south-west and north-east monsoon currents. The south-west monsoon current, striking the west side of the mountain range, gives heavy rainfall during the months of June to September in the south-west quadrant of the island. The north-east monsoon rainfall (October to February) is not only less heavy, but is more evenly distributed throughout the island. It thus comes about that the south-west quadrant receives rainfall during both monsoons—mean rainfall 75 to 200 inches—and it is in consequence known as the Wet Zone, whilst the rest of the island (low-lands) receives rainfall only during the north-east monsoon (average annual rainfall 25 to 50 inches), and it is known as the Dry Zone. In the Wet Zone there is normally an appreciable rainfall every month of the year whilst in the Dry Zone there is usually a prolonged drought from May to October.

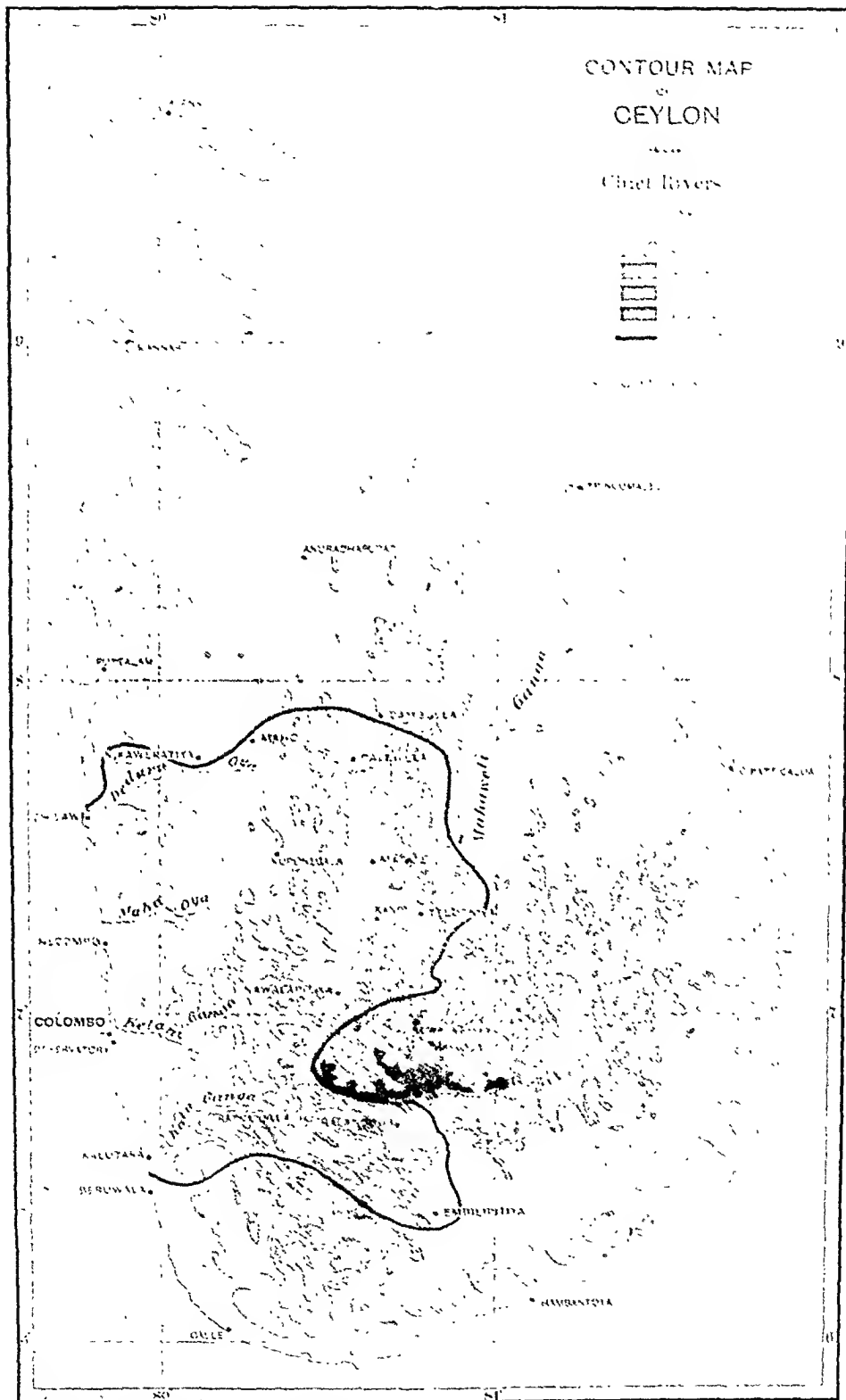
Population.

The population of the island in 1931 was 5·3 millions, which represents the increase of 17·9 per cent. during the decade 1921–1930. The mean density of the population is 208 per square mile, but it varies greatly, the density being greatest in the south-west quadrant and in the Jaffna peninsula. Most of the population is concentrated in the south-west quadrant, and it is in this area that the tea, rubber and coconut estates, upon which its prosperity largely depends, are mainly situated.

Figure 2 shows the relative salubrity of the twenty-one districts of the island, as measured by the mean natural increase of the population over a period of 30 years (1901–1930). The districts vary so greatly in salubrity that the island can readily be divided into three health zones in accordance with the excess of births over deaths. The details in respect of these three zones are as follows:—

	Area.	Popula- tion.	Birth- rate.	Death- rate.	Infantile Mortality- rate.	Natural Increase.	Population Under 4 Years per 1,000.
	Per cent.	Per cent.					
Zone A	24	69	39·3	25·5	164	+ 13·8	148
Zone B	48	27	42·5	35·7	235	+ 6·8	151
Zone C	28	4	37·2	41·6	338	— 4·4	113

FIG. 1.



Note.—The thick black line encloses the catchments of the four rivers, Deduru Oya, Maha Oya, Kelani Ganga, and Kalu Ganga, and the upper reaches of the Mahaweli Ganga.

(After Briercliffe.)

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FIG. 3

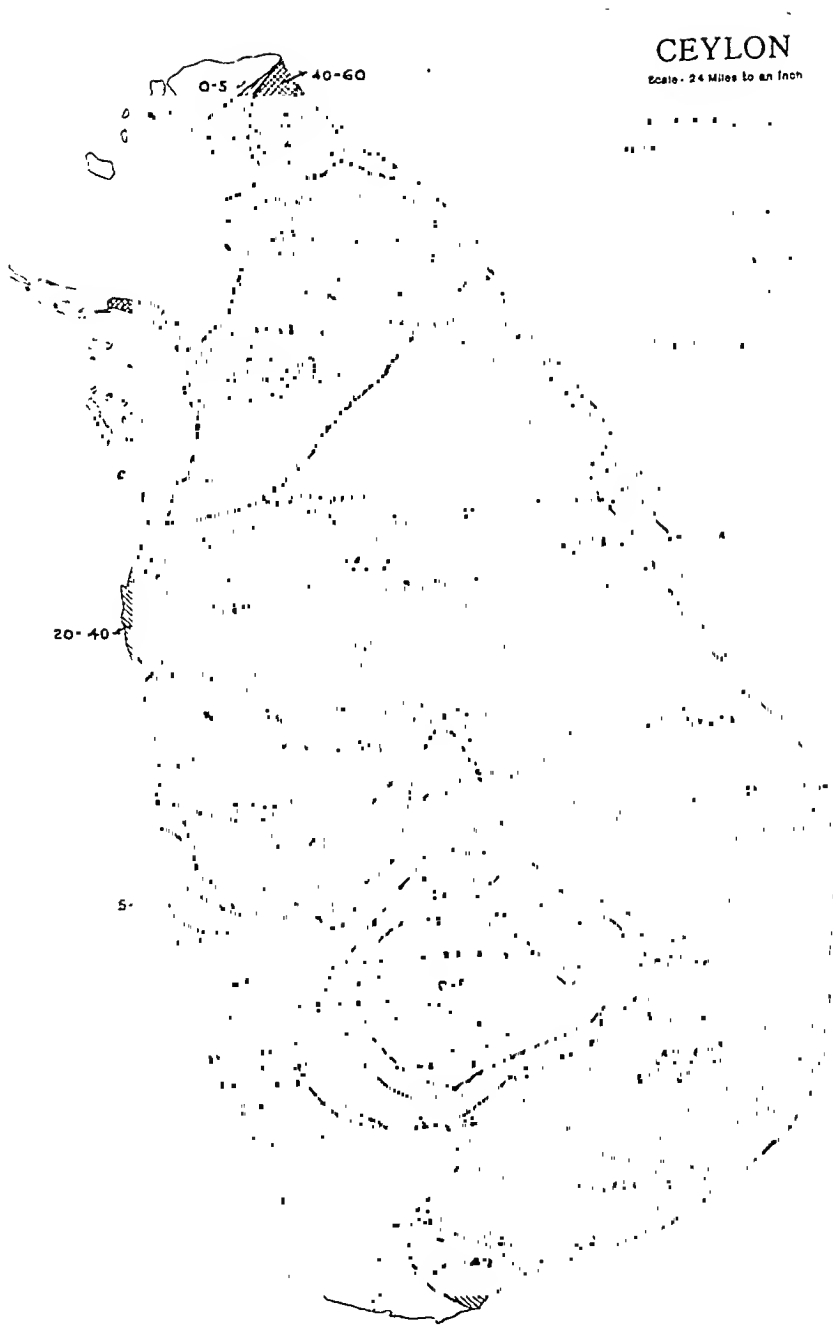


Fig. 3 (p. 435) shows the incidence of endemic malaria, as determined by Mr. H. F. CARTER, Medical Entomologist, in 1922 and 1923. It will be seen that the zonal distribution of the spleen-rate bears a striking relation to the health zones. Thus, in the south-west quadrant of the island, and in the Jaffna peninsula, the spleen-rate is almost everywhere extremely low (0-5 per cent.). Other noteworthy features are the narrow belt in the sub-montane traet with the spleen-rate of 10-20 per cent., the broader belt with the spleen-rate of 20-40 per cent., the northern boundary of which corresponds closely with the northern limit of the Wet Zone, and the extensive area in the northern half of the island, and in the south-east quadrant, with a spleen-rate of 40-80 per cent. or more.

It is not open to doubt that the main cause of the extreme insalubrity of Zone C (and to a less extent of Zone B) is the high incidence of endemic malaria, and to this disease must be attributed, in very large measure, the relatively and absolutely high death-rate and infantile mortality-rate, the relatively small proportion of young children, and the decline of population in Zone C.

(B) GENERAL REVIEW OF THE EPIDEMIC.

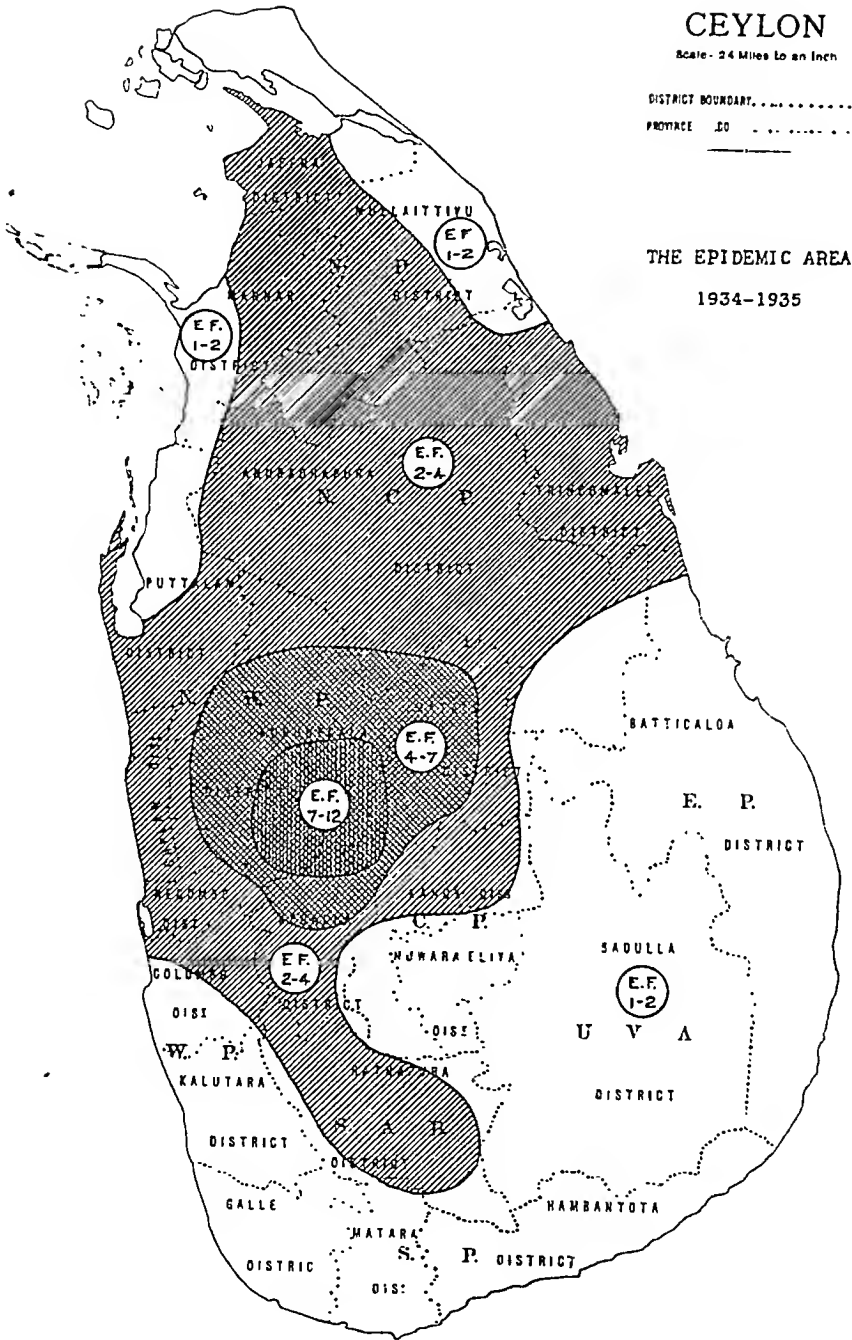
(1) *Spatial Distribution.*

The distribution of the epidemic, as judged by mortality during the months of November, 1934, to March, 1935, is shown in Fig. 4. The shading depicts its intensity, as measured by the "epidemic figures" of registration circles, which represent the number of times the mortality exceeded the normal during the above period. It will be seen that the epidemic exhibited the focal character described by CHRISTOPHERS. The central nucleus comprised five adjoining registration circles in three districts with "epidemic figures" of between 7 and 12. It is surrounded by a larger area containing ten registration circles with "epidemic figures" of 4 to 7, which, in turn, is encircled by a larger area extending broadly to the north, and in a tongue-like projection towards the south, with "epidemic figures" of 2 to 4. It will be observed that the configuration of the main epidemic area is not in strict conformity with a distribution based upon the five rivers. It will also be noted that the epidemic area bears a rough relationship to the area with a moderate spleen-rate.

The morbidity associated with the epidemic, as measured by hospital attendances, followed generally the distribution of mortality, and it may be said that, although intense epidemic conditions were confined to a restricted area—the main epidemic area—scarcely any portion of the island failed to show some increase of morbidity and mortality during the epidemic.

The onset of the epidemic was heralded by a slight increase of hospital attendances late in September and early in October at a few dispensaries on the banks of the Maha Oya, but the sudden sharp rise which marked the onset of the epidemic commenced in the last week of October in the north of the

FIG. 4.



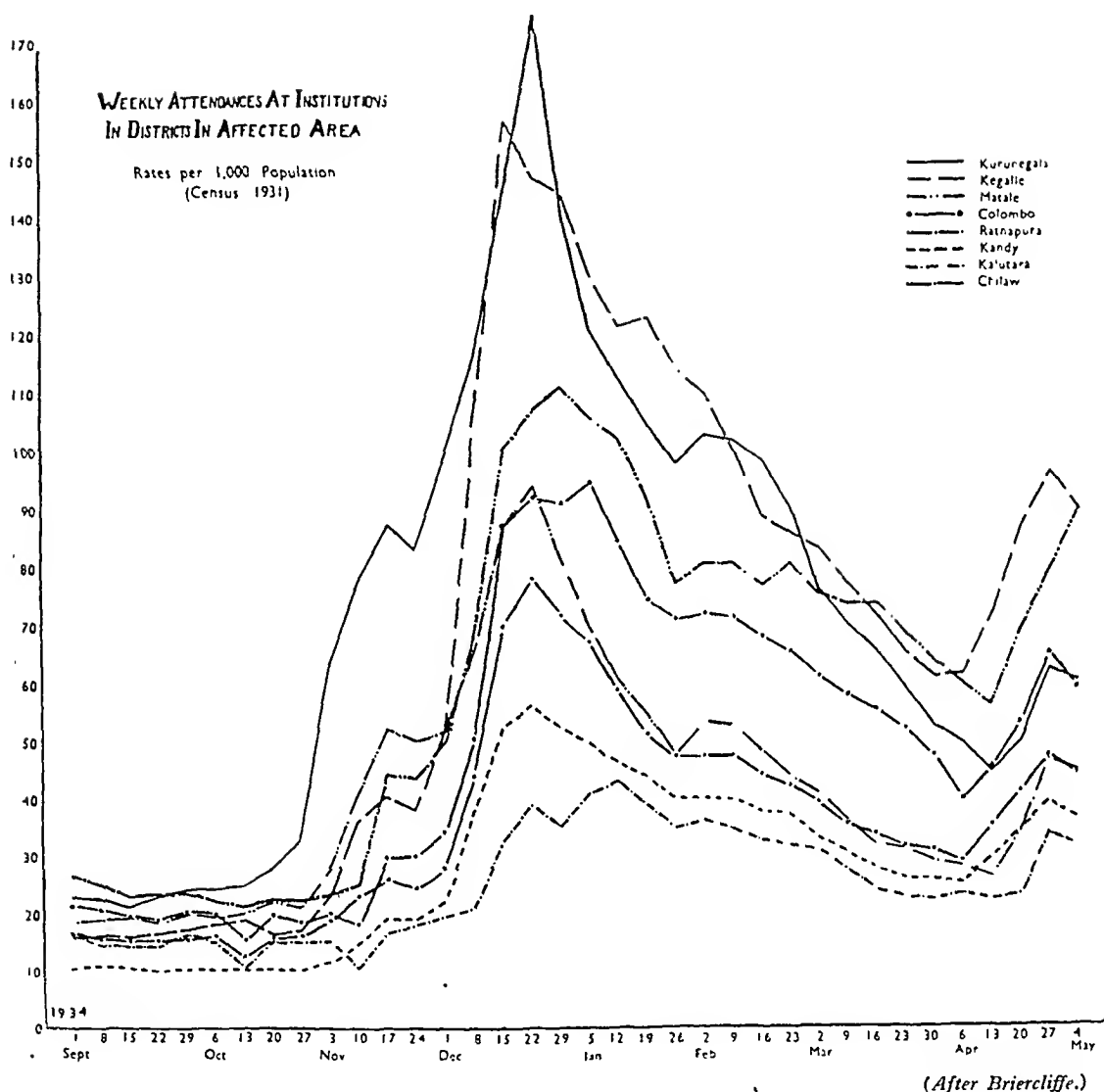


FIG. 5 (p. 446-448).

The chart displays the following features :—

(1) The onset of the epidemic about one month earlier in the north of the epidemic area than in the south. The only exception is the district of Kandy, which, however, is not in the plains but in the sub-mountain tract.

(2) In spite of the varying intensity of the epidemic, the epidemic wave (morbidity) exhibited everywhere, although in a blurred form, four peaks, the second being the largest.

(3) The magnitude of the second wave—the first wave of new infections—was everywhere roughly proportionate to the magnitude of the first wave.

(4) The commencement of the second wave in April, 1935, about 24 weeks after the commencement of the first wave in November, 1934.

(5) The varying intensity of the epidemic is indicated by the epidemic figures (E.F.) of the eight districts as follows :—Kurunegala, 5.7 ; Kegalle, 5.0 ; Matale, 4.4 ; Colombo, 1.7 ; Ratnapura, 2.2 ; Kandy, 2.9 ; Kalutara, 1.2 ; and Chilaw, 2.9.

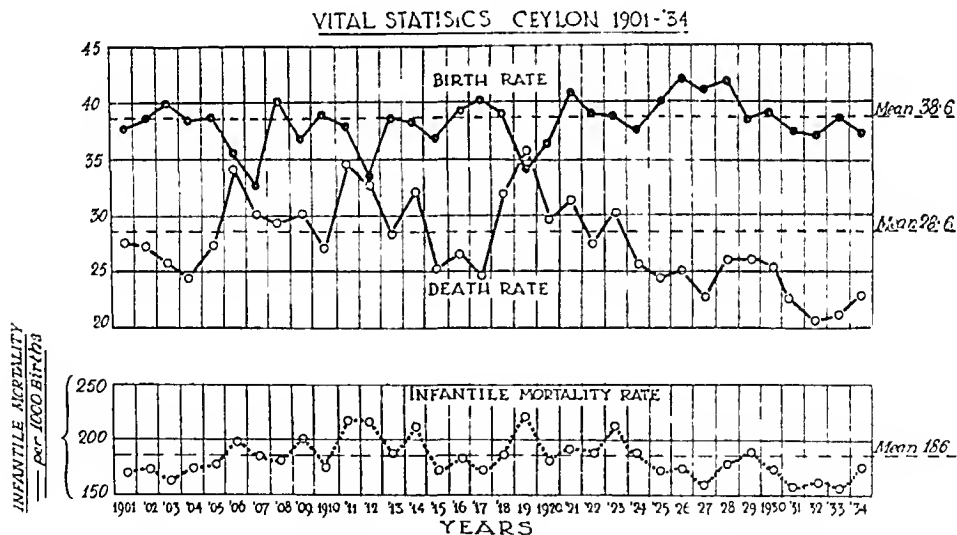
epidemic area, and somewhat later, as shown in Fig. 5, in the south of the epidemic area, in which it did not commence until early in December.

The mortality associated with the epidemic exhibited no abnormal features in respect of age incidence, and it will suffice to remark that during the acute stage of the epidemic infants and young children were mainly involved, whilst towards the decline of the epidemic the mortality was almost exclusively confined to the 40-60 age-group and to persons over 60 years of age.

(2) Cyclical Periodicity.

Curves representing the death-rate, birth-rate and infantile mortality-rate during the period 1901-1930 are shown in Fig. 6. It will be observed that a period of 4 years of exceptionally good health preceded the outbreak of the epidemic. This circumstance was associated with a low incidence of endemic malaria and the absence of any epidemic. It may be presumed therefore that in October, 1934, the spleen-rate was probably everywhere lower than normal, and that the proportion of children per thousand of population was somewhat higher than usual.

FIGURE 6.



The chart also shows the occurrence periodically of a conspicuous rise of the curves representing the death-rate and the infantile mortality-rate, together with an equally conspicuous fall of the birth-rate in the year following each sharp rise of the death-rate. This combination of circumstances is characteristic of regional epidemics of malaria, and, as the contemporary records show that malaria was widely prevalent in these years, it may confidently be stated that,

except in the year 1918 (when influenza was pandemic) and possibly 1919, the high death-rate in the years 1906, 1911, 1914, 1919(?), 1923, and 1928 was mainly caused by malaria in epidemic form. During the period 1901-1930 malaria epidemics therefore occurred in Ceylon at intervals of approximately 5 years, and on this basis another epidemic might be regarded as due about the year 1934.

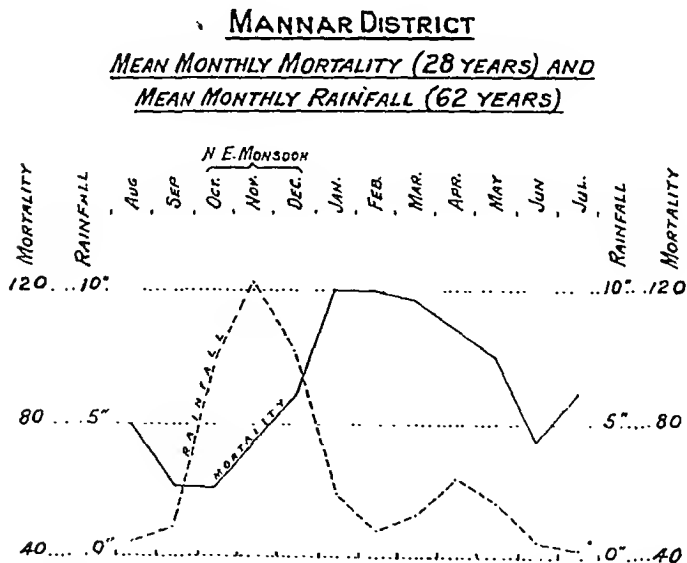
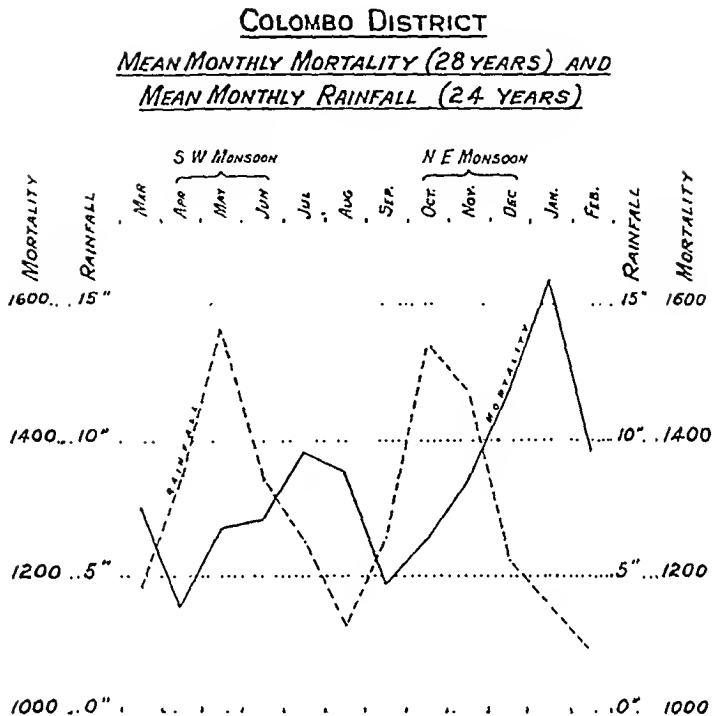
(3) *Seasonal Periodicity.*

In most countries there is only one malaria season, and malaria epidemics, when they occur, invariably take place at that period of the year recognized to be the "malaria season." An analysis of the mean monthly incidence of mortality in each of the twenty-one districts during the period 1901-1930 shows that in the Wet Zone of Ceylon there are two malaria seasons each year, one in the summer and the other in the winter, whilst in the Dry Zone there is only one malaria season. In other words, where there are two rainy seasons there are two malaria seasons, and where there is only one rainy season there is only one malaria season. This fact is illustrated in Fig. 7, which shows, in respect of one representative district in the Dry and Wet Zones respectively, the malaria season of each zone, and its close relation to the rainy season. As the epidemic wave is an exaggeration of the normal seasonal wave it would be expected that epidemics would occur in the Wet Zone both in the summer and in the winter, and that in the Dry Zone epidemics would occur in the winter alone. This expectation is fulfilled. It will be seen from Figs. 8 and 9 that the epidemics in the years 1906 and 1911 exhibit well-marked waves in the summer and winter, and it will also be noted that the first wave occurred in the summer. On the other hand, in the case of the epidemic in 1934 (Fig. 10), the first wave commenced in the winter. These were Wet Zone epidemics and there has been no great epidemic in the Dry Zone during the period under review, but the annual epidemic in this zone is usually so marked as almost to constitute an annual epidemic. The seasonal wave of malaria and the seasonal periodicity of epidemics in Ceylon present therefore some unusual features.

(4) *Relation to Drought and Rainfall.*

Just as it was generally recognized in the Punjab long before it was proved to be a fact by CHRISTOPHERS that malaria epidemics in Northern India were determined by excessive monsoon rainfall, so in Ceylon it has long been known to medical men and laymen alike that a wet year in the Wet Zone is a healthy year, and that in this zone a failure of the monsoon is almost invariably followed by an unusual prevalence of malaria. In order to determine with precision the nature of this relationship a statistical analysis was undertaken of factors representing "rainfall" and "malaria" in eight representative districts of the island over a period of 34 years (1901-1934). The result of this analysis permits of the inference that in the Wet Zone a deficiency of rainfall in the spring is favourable to the occurrence of a summer epidemic, and that a deficiency of rainfall during

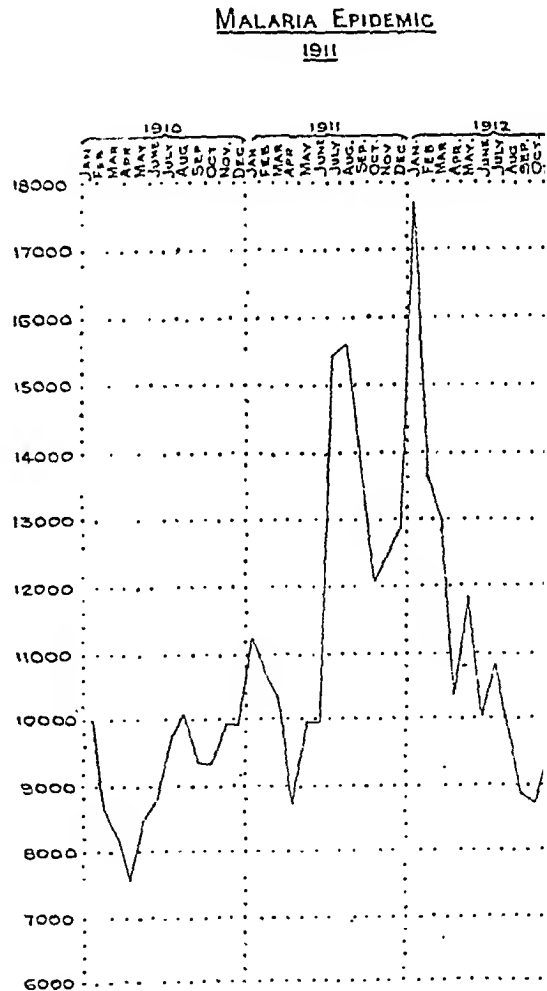
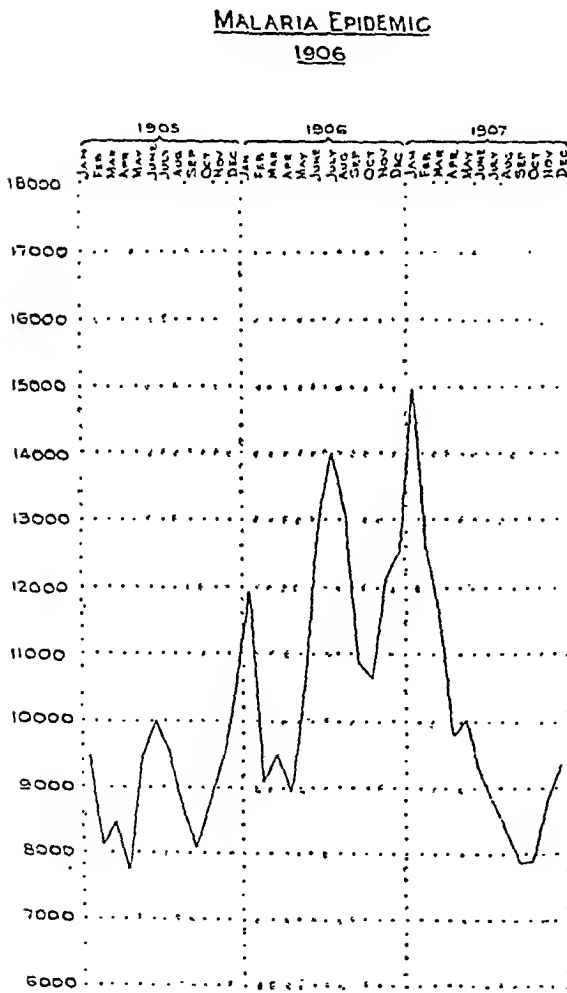
FIG. 7.



July, August and September (south-west monsoon) is favourable to the occurrence of a winter epidemic. On the other hand in the Dry Zone, as represented by Trincomalee, excessive rainfall during the winter is favourable to the occurrence of a winter epidemic. The malaria epidemic of 1934-1935 was a Wet Zone epidemic associated with the greatest drought on record during the months of July to September. The drought ceased early in October, but, although the rainfall of October was heavy (11.3 inches at Kurunegala), it was nearly everywhere below normal in the epidemic area (-4.5 inches at Kurunegala), and it remained below normal for several months thereafter.

FIG. 8.

FIG. 9.

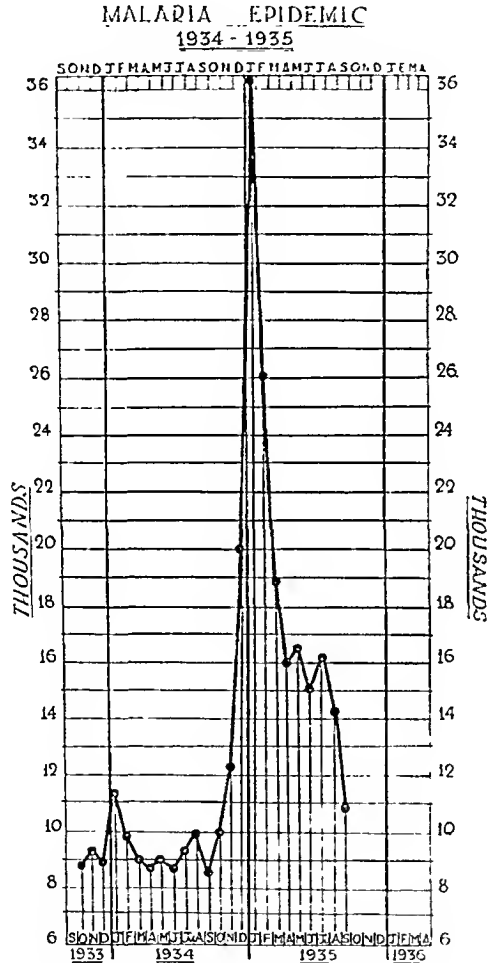


The curves in Figs. 8, 9 and 10 show the total deaths in Ceylon by months during the period indicated.

(5) *Relation to Anophelines.*

The anopheline fauna of Ceylon and the relation of each species to malaria has formed the subject of close study by Mr. H. F. CARTER, Medical Entomologist, during the past 12 years. He has shown that, although other carrier species occur in Ceylon, only one species, *A. culicifacies*, is of any importance so far as

FIG. 10.



malaria is concerned. He regards *A. culicifacies* as essentially a Dry Zone species, since it is widely prevalent in the north, east, and south-east of the island, and in the sub-montane tract at elevations below about 2,000 feet. In the Wet Zone, however, this species appears to be much less prevalent, whilst in the area intermediate between the Dry and Wet Zones its prevalence is variable from

year to year, being unusually abundant in the north of the Wet Zone in years of drought. This feature was particularly conspicuous during the recent epidemic, when *A. culicifacies* was extremely prevalent much to the south of its normal habitat, more particularly in the neighbourhood of the rivers Deduru Oya, Maha Oya, Kelani Ganga, Kalu Oya, and in the upper reaches of the Mahaveli Ganga. No observations appear to have been made in the river beds during the drought, but in the month of November, 1934, and throughout the epidemic, *A. culicifacies* was extremely prevalent throughout the catchments of these rivers, and one of its favourite breeding places was the pools and the sheets of water in the beds of these rivers and their main branches. This fact led many to conclude that the abnormal prevalence of this species in the epidemic area was the main cause of the recent epidemic, more especially as it showed an extremely high infection-rate (12.9 per cent. in December, 1934) during the epidemic.

(6) *Relation to Malaria Parasites.*

The observations made during the course of the epidemic suggested that the benign tertian parasite played a predominant part during the early stages of the epidemic, but gave place to the malignant tertian parasite towards its close. These observations are in general conformity with Indian experience, except that a relatively larger share would appear to have been taken in Ceylon by the benign tertian parasite at all stages of the epidemic.

No observations were made in regard to the frequency and intensity of infections during the acute stage of the epidemic, and no remarks can be made on this subject.

4.—THE MECHANISM OF THE EPIDEMIC WAVE.

(A) THE PRIMARY WAVE.

The difficulty of accounting for the sudden and widespread outbreak of sickness in association with malaria epidemics at a time when the spleen-rate is extremely low and when malaria parasites, more particularly gametocytes, can scarcely be detected in the peripheral blood, even in thick films, pointed to the necessity of investigating closely the happenings during the early stage of the epidemic.

As the first step in this direction it was necessary to determine, as precisely as possible, the date of onset of the epidemic, and with this object in view the attendance registers of a large number of hospitals throughout the epidemic area were scrutinised and analysed. These observations brought to light the fact that not only was the onset of the epidemic remarkably abrupt but that it could often be fixed to a day. Thus, at the Civil Hospital, Kurunegala, the daily average number of attendances, which had fluctuated between 130 and 150 per diem for several months before the epidemic, was more than

doubled on 29th October, 1934, with the result that the total attendances, which in the week ending 27th October were 1,305, numbered 2,763 at the end of the following week. The actual figures, in respect of *malaria cases only*, for the last 8 days of October were as follows :—

October 24th	74	October 28th	106
„ 25th	80	„ 29th	306
„ 26th	112	„ 30th	262
„ 27th	121	„ 31st	310

The figures for total attendances at other hospitals and dispensaries in the epidemic area showed similar features, although, as shown in Fig. 5, the epidemic commenced about one month earlier in the north than in the south of the epidemic area. The onset of the epidemic was therefore even more abrupt than would be gleaned from a scrutiny of the weekly returns, unless the day of the onset of the epidemic happened to coincide with the first day of the week.

For the purpose of the study of the epidemic wave it was decided to confine attention to a single locality, and Kurunegala town, population 10,500, was selected for this purpose, partly because it was the most severely affected large town in the island, and partly because exceptionally complete statistical data were obtainable. It should be explained that no burial can take place in this town until the death has been registered, and all deaths are consequently registered with extreme promptitude. In order, however, to ensure absolute accuracy in respect of the date of death—a matter of some importance—every death that occurred during the nine months from September, 1934, to May, 1935, has been relegated to the week in which it actually occurred, as opposed to the week in which it was registered. The total number of deaths each week during the months of September, 1934, to May, 1935, together with the deaths under 10 years and over 10 years of age respectively, are shown on Fig. 11, in which the weekly total attendances at the Civil Hospital, Kurunegala, are also depicted in the form of a curve.

The Morbidity Wave.

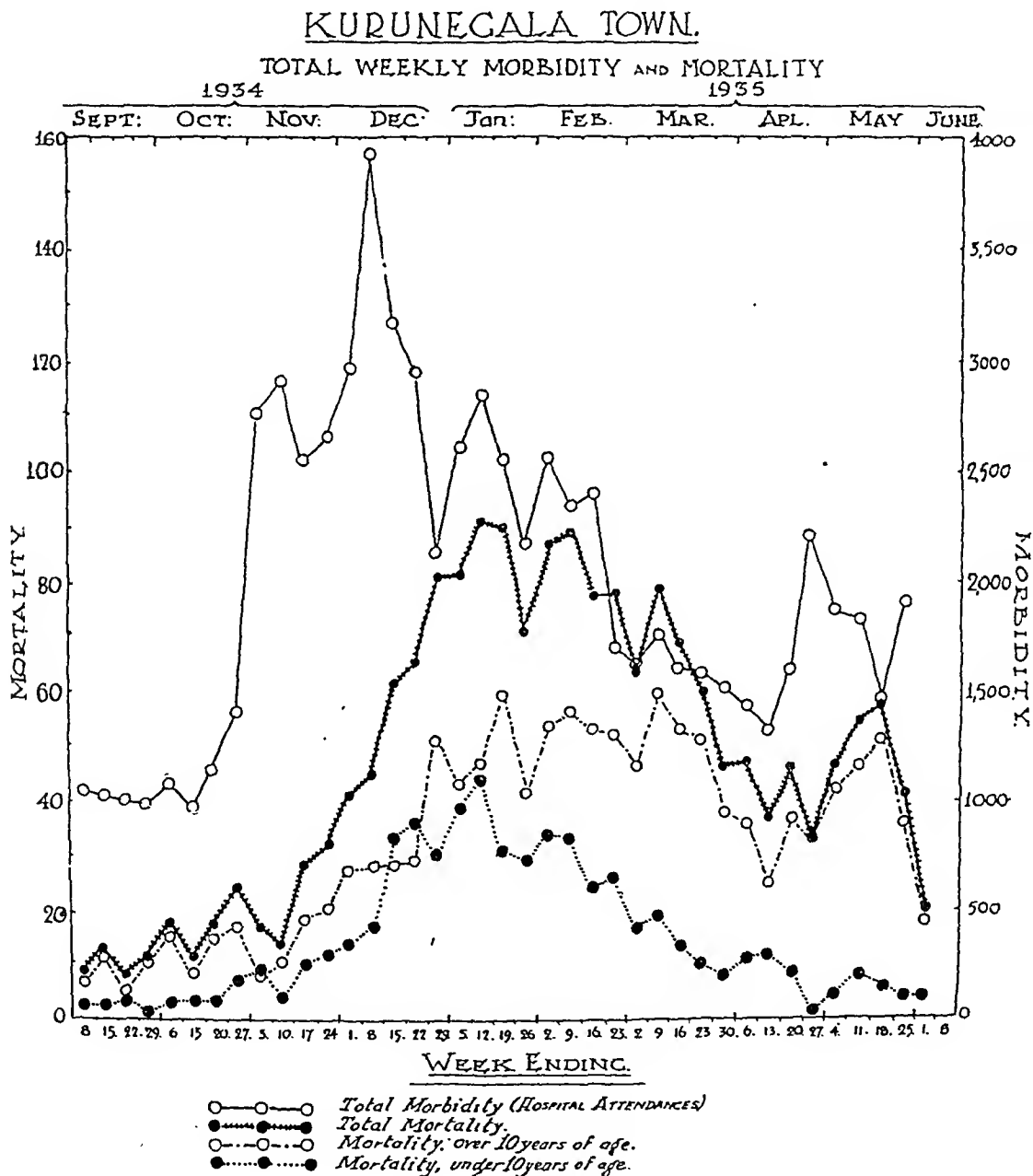
The curve representing morbidity shows a primary wave lasting from November to February and a second wave commencing in the middle of April. The primary wave, it will be seen, has four peaks, of which the second is much the most conspicuous. The first peak occurs in the week ending 10th November, the second in the week ending 8th December, the third in the week ending 12th January, and the fourth in the week ending 2nd February, in other words there are four waves, the first three at intervals of approximately one month.

That these features are not peculiar to the epidemic at Kurunegala, but are characteristic of the whole epidemic is suggested by the fact that they are apparent, although in a blurred form, in the curves of the whole epidemic

depicted in Fig. 5. These four waves are also present in the charts prepared by CHRISTOPHERS, GILL, and COVELL in respect of malaria epidemics in India. They are discernible, for example, in Fig. 12, which has been prepared from data given by COVELL and BAILY (1932) in respect of the malaria epidemic in Northern Sind in the year 1929.

It therefore seems permissible to conclude that the primary wave of

FIG. 11.

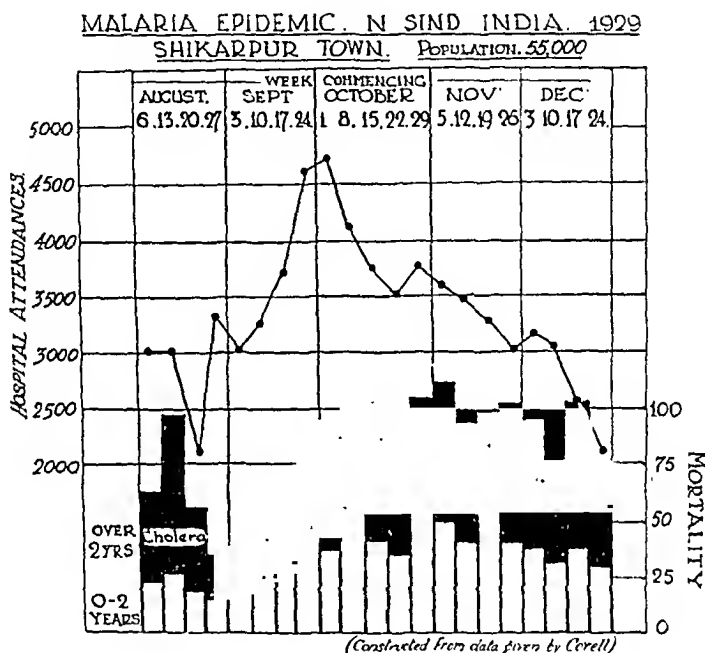


morbidity associated with malaria epidemics is characterized by four waves, the second of which is the largest, at intervals of approximately 4 weeks.

The Mortality Wave.

The curve representing total mortality differs from the morbidity curve both in general configuration and in the number of peaks. It will be seen

FIG. 12.



that there are only three peaks of mortality at intervals of about 4 weeks, so that the primary wave of the epidemic comprises four subsidiary waves of morbidity at intervals of about 4 weeks and three subsidiary waves of mortality at the same intervals.

The most striking difference between the curves representing morbidity and mortality is the abrupt rise of the morbidity wave and the more gradual rise of the mortality wave, so that the latter does not reach its acme until about 10 weeks after the onset of the epidemic.

The curve representing total mortality shows a small rise in the third week of the epidemic and rises slowly thereafter until the 7th week when a sharp rise occurs. It will be seen that this sharp rise is mainly due to mortality in the 0-10 age-group, but the most striking feature exhibited by the mortality statistics is the absence of any appreciable increase of mortality amongst infants and children until the 7th week of the epidemic.

The morbidity and mortality curves in the Sind epidemic (Fig. 12) exhibit

almost precisely similar features, and it therefore seems permissible to infer that the form and character of the waves of morbidity and mortality during the malaria epidemic at Kurunegala are typical of all malaria epidemics.

It is now proposed to consider the interpretation to be placed upon these facts.

The long lag—about 6 weeks—between the onset of the epidemic, as determined by the first sharp rise of morbidity, and the first rise of mortality in the 0–10 age-group, would appear to imply either that no new infections occurred amongst children during the early stages of the epidemic, or that the infections were so mild at this period as not to cause death for a period of nearly 6 weeks.

In the latter case it would be expected that some of the children at least would have been sufficiently ill to have attended hospital, and that, in consequence, there would have been an appreciable increase in the percentage of children amongst the patients attending hospital for malaria immediately after the onset of the epidemic.

The attendances for malaria at a number of hospitals and dispensaries were accordingly examined by age-periods for short periods *before* and *after* the onset of the epidemic. The result of making these calculations is illustrated in Table 1.

It will be seen that no appreciable change occurred in the age-distribution of the malaria cases in association with the onset of the epidemic; in fact, at the Civil Hospital, Kurunegala, the outbreak of the epidemic was followed by a small but appreciable decrease in the proportion of the patients belonging to the 0–4 age-group and a corresponding increase in the 21–40 age-group.

It must therefore be concluded that the children, if sick, were either not sufficiently ill to attend hospital or were too ill to do so. The latter is an untenable proposition, since some at least would have died, whilst it has been shown that

TABLE I.

ANALYSIS BY AGE-PERIODS OF PATIENTS ATTENDING HOSPITAL FOR MALARIA SHORTLY BEFORE AND AFTER THE ONSET OF THE EPIDEMIC.

Age Period.	Alawwa Dispensary.		Polgahawela Dispensary.		Kurunegala Hospital.	
	Before Onset (14–21 Sept.).	After Onset (21–28 Sept.).	Before Onset (9–13 Oct.).	After Onset (14–18 Oct.).	Before Onset (22–25 Oct.).	After Onset (29–31 Oct.).
0–4	27.5	26.4	34.5	31.9	24.4	19.7
5–10	23.0	17.6	16.2	18.3	20.0	20.4
11–20	21.2	21.2	16.2	18.7	19.0	20.0
21–40	21.0	24.0	26.3	23.8	27.4	31.4
41–60	6.2	8.9	5.5	5.8	7.6	7.1
60 +	1.0	1.8	1.2	1.2	1.4	1.3
Total	240	421	365	495	273	874

no increase in the number of deaths of children under 10 years of age occurred during the first 5 weeks of the epidemic. Alternatively, the children were ill, but, although not treated, did not die for about 6 weeks. The adoption of this hypothesis is difficult, since it is well known that children usually succumb rapidly to primary attacks of malaria, more especially during epidemics, but to verify this point an analysis was undertaken of the case-sheets of all the fatal cases of uncomplicated malaria (113 in number) in children under 5 years of age, who were treated in 35 hospitals, mainly in the epidemic area, during the months of November and December, 1934. It was found that the average duration of the fatal illness (including the period spent in hospital) was 8 days, or 6 days in the case of 49 children (43 per cent. of the total) admitted with convulsions or cerebral malaria. These patients had the benefit of skilled treatment and nursing, and it seems probable therefore that the average duration of the fatal illness in untreated cases of acute malaria in children under 5 years of age during the early stages of the epidemic would not exceed one week.

In these circumstances the conclusion is inevitable that the children must have escaped infection almost completely during the first 4 weeks of the epidemic, and, if this be so, it is difficult to believe that the morbidity amongst adults during this period could have been mainly caused by new infections, since it would imply that infected anophelins had selected adults alone for attack.

Furthermore, to permit of an outbreak of sickness due to new infections over a wide area at the end of October, it would be necessary to assume that a large number of anophelins had become infected from such human carriers as existed during a period of good health, and had thereafter dispersed, and, in due course, infected a large number of persons (*excluding children*) about the same time, who, after an identical incubation period, commenced to fall ill together.

This sequence of events is too improbable to call for serious consideration, and it is therefore held that the sickness during the first month of the epidemic was mainly due to an "epidemic of relapses" amongst apparently healthy human carriers.

On this basis the happenings during the epidemic wave can readily be interpreted. The absence of a marked change in the age-distribution of the patients at the outbreak of the epidemic would appear to indicate that the relapses were a reflection of the pre-existing proportion of human carriers in each age-group. Again, if the first morbidity wave was due to relapses, the first crop of new infections—the second morbidity wave—would be expected about 1 month later, allowing 10 days for the appearance of mature gametocytes in the peripheral blood, 10 days for the mosquito-cycle, and 10 days as the incubation period in man. Similarly, a third wave and a fourth wave would be expected to occur, successively, at intervals of about 1 month, but the removal by death of the most susceptible and the rising immunity of the community would tend to reduce the magnitude of each successive wave, so that the first wave of new infections—the second morbidity wave—would be the highest.

Then again, if the first morbidity wave was due to relapses, the absence of any appreciable mortality amongst children during the first 4 weeks of the epidemic can readily be understood. On this view the association of three waves of mortality with four waves of morbidity is due to the absence of a mortality wave in association with the first morbidity wave.

The first sharp rise in mortality in the 7th week of the epidemic, which is largely due to deaths amongst children, would thus be associated with new infections contracted in the 5th and 6th weeks, this time interval being in accordance with expectations based upon the analysis of the hospital statistics, an illness of 1 week's duration, on an average, being allowed in the case of children, and 2 weeks in the case of adults.

In the light of this interpretation, the mortality wave which reached its acme in the middle of January would reflect new infections contracted during the month of *December*, whilst the second mortality wave in February and the third mortality wave in March would reflect the mortality associated with the third and fourth morbidity waves in January and February respectively.

No observations were made upon the behaviour of the parasite and the spleen during the epidemic, but COVELL and BAILY (1932) found that a marked rise in the number and intensity of infections occurred during the 13th week (corresponding to the third morbidity wave) of the epidemic in Northern Sind, which they attributed to the early relapse of malignant tertian infections. They also regarded these relapses as responsible for a change in the frequency-distribution of enlarged spleens, whereby a uni-modal distribution in the 7th week of the epidemic became bi-modal in the 13th week.

These observations are, however, susceptible of an alternative interpretation; for, in accordance with CHRISTOPHERS' (1924) "theory of the splen-rate," one crop of new infections (one *splen*) would give rise in a community of non-immune children to a uni-modal frequency-distribution of enlarged spleens, since, in accordance with the law of probability, the infections would not be evenly distributed, some children receiving 0, 1, 2, 3, etc., *splens* respectively. If, however, one month later another crop of new infections be superimposed on this community, the result would be the replacement of the uni-modal by a bi-modal frequency-distribution of enlarged spleens.

The changes observed by COVELL and BAILY in the number and intensity of infections and in the frequency-distribution of enlarged spleens during the acute stage of the epidemic in Sind are therefore in accordance with what would be expected on the basis of the present interpretation of the happenings during the epidemic wave.

In the light of the assumption that the first morbidity wave was caused by relapses, the form of the morbidity and mortality wave, the association of four subsidiary waves of morbidity with three waves of mortality, and the apparent lag of the mortality wave become readily explicable, but the most striking feature of this interpretation is that it solves the difficulty associated

with the sudden and widespread outbreak of malaria epidemics at a time when there is a great scarcity of gametocytes in the peripheral blood of human carriers.

To account for this anomaly, MACDONALD and MAJID (1931) suggested that, if the period of transmission were sufficiently prolonged, a large gametocyte population would eventually be created by a process of geometrical progression; and this view was adopted by COVELL and BAILY (1932), who remarked that, if it be assumed that one crescent carrier can give rise to ten crescent carriers after the lapse of one month, an increase in the number of crescent carriers from 5 per cent. in the 5th week of the epidemic to 50 per cent. in the 9th week would be possible. This explanation, however, fails to account for the events during the *first* week of the epidemic, and a slow geometrical increase in the number of carriers is not capable of accounting for the sudden and widespread outbreak of sickness during the first week.

(B) THE SECOND WAVE.

The first wave was succeeded by a second wave in April, shortly after my arrival in Ceylon. At this time I assumed, in accordance with Indian experience, that there would be no second wave, but this assumption proved to be wrong, and the prediction of S. P. JAMES (1935) that a second wave would occur in the spring was borne out by the event.

The second wave affected the whole epidemic area, as is shown in Fig. 5, and it will be seen that, although of smaller magnitude than the first wave, it exhibited generally the same relative intensity in different parts of the epidemic area as the first wave. The second wave in Kurunegala town (*vide* Fig. 11) commenced in the middle of April or about 24 weeks after the initial explosion. I was touring in the epidemic area at this time and I was consequently able to investigate the circumstances attending its onset. It was found that the morbidity wave commenced almost as abruptly and was almost as marked as the primary wave; thus, the attendances at five hospitals visited on 20th April, 1935, were as shown in Table II.

TABLE II.
TOTAL ATTENDANCES. 10TH-19TH APRIL.

Locality.	10th.	11th.	12th.	13th.	14th.*	15th.	16th.	17th.	18th.	19th.
Matale	178	192	191	113	—	195	237	319	277	328
Teldeniya	204	177	201	130	27	156	181	248	233	237
Kandy	49	63	42	49	28	101	89	109	92	107
Gampola	274	316	333	295	62	424	396	380	274	261
Nawalapitiya	369	354	279	377	108	464	398	477	470	404

* Buddhist New Year.

It is thus clear that the onset of the second wave in the area served by these five hospitals took place on 15th April, 1935. Many opinions were held in regard to its cause. Some medical officers in charge of hospitals attributed it to the normal seasonal wave due in this area in April, others considered it to represent relapses, whilst others thought that it was due in part to new infections associated with recent rainfall or with a preceding period of drought, but nearly all agreed that no appreciable change in clinical characters occurred in association with the onset of the wave, and many stressed the difficulty of distinguishing fresh infections from relapses owing to the fact that many of the patients gave a history of repeated attacks of fever during the preceding 5 months.

House to house visits made in villages brought to light the fact that, although many adults were sick, there were few serious cases amongst either adults or children, and there had been no recent increase of deaths. Relatively few children suffering from fever were seen, although the spleen-rate of groups of children was as high as 98 per cent. The conclusion reached and recorded at the time was that probably most of the patients were suffering from relapses, but that an appreciable number of new infections were occurring in the vicinity of Gampola and Nawalapitiya. Later, when it became known that the morbidity wave was associated with an appreciable rise of the infection-rate of *A. culicifacies*, and was followed by a wave of enhanced mortality, it was realized that the wave was a true second wave, and it is now regarded as an exact replica on a smaller scale of the primary wave, and as being initiated, like the first wave, by a wave of relapses. The resemblance is even closer than the chart shows since the morbidity wave in April was followed by a second wave in June, each wave being associated, at an interval of about 2 weeks, by a small wave of enhanced mortality. The main difference between the primary and second wave is that an interval of about 6 weeks elapsed between the first rise of morbidity and the first appreciable rise of mortality in November, 1934, whilst in April, 1935, the interval was only about 2 weeks; but this is in accordance with expectations, since there was a widespread human reservoir of infection in April, 1935, but not in October, 1934.

5.—THE NATURE AND SIGNIFICANCE OF THE EPIDEMIC POTENTIAL.

The study of the epidemic wave has been the means of bringing to light much evidence pointing to the conclusion that two factors of an entirely different order are concerned in the production of the epidemic wave.

The first in order of importance, as well as in precedence, is concerned with a change in the relationship of the malaria parasite and the human host, whereby, at the commencement of an epidemic, an "epidemic of relapses" is precipitated amongst human carriers.

Whatever the nature of this factor may be it clearly has nothing to do with the *immediate* presence of anopheline mosquitoes or with the abundance of their breeding places. It is furthermore clear that this factor plays a part

of fundamental importance in the mechanism of the epidemic. It thus determines the magnitude of the initial explosion, which, in turn, is largely responsible for the magnitude of the successive waves.

The second factor in the production of the epidemic wave is concerned with the quantitative relationship existing between "infection" and "immunity"; in other words, it is concerned with the number of anophelins capable of transmitting infection and the number of non-immunes capable of being infected.

The magnitude of an epidemic, it may be presumed, will depend upon the occurrence in favourable conjunction of both these factors, and it is reasonable to suppose that, if the environmental conditions are not favourable to transmission, the two factors may be dissociated, and an epidemic of relapses will then not be followed by a wave of new infections. It is possible that the "spring rise" of malaria in Northern India, and the infections which occur in Europe in the spring, known as "delayed primary infections" and "recurrences," may be explained on these lines.

But whether this surmise be correct or not, it is obviously a matter of extreme importance to determine the cause of the "epidemic of relapses," which it seems probable is "the unknown influence," "the epidemic influence," and "the epidemic potential," long suspected to be the determining cause of epidemics.

The widespread and almost simultaneous outbreak of malaria epidemics and their peculiar focal character suggested to CHRISTOPHERS (1911), the intervention of "some general determining cause," and it is therefore possible, in view of the close association of the rainfall season with the malaria season, that a causal relationship may exist between atmospheric humidity and the epidemic of relapses that initiates an epidemic.

It is moreover known, as the result of the study of malaria epidemics in Northern India by GILL (1921, 1928), COVELL (1932) and others, that malaria epidemics are invariably associated with high atmospheric humidity during the pre-epidemic period, but it has hitherto been supposed that high atmospheric humidity favoured the occurrence of an epidemic by reason of its influence upon the longevity and metabolic activity of the insect-carrier, although GILL (1928) suggested that possibly atmospheric states might also exercise some influence upon the malaria parasite during its intracorporeal phase.

The Ceylon epidemic was preceded by a sharp rise of relative humidity during the pre-epidemic period. This fact is illustrated in Fig. 13, where the mean weekly atmospheric humidity, expressed in the form of saturation deficiency, is shown for the period of the epidemic.

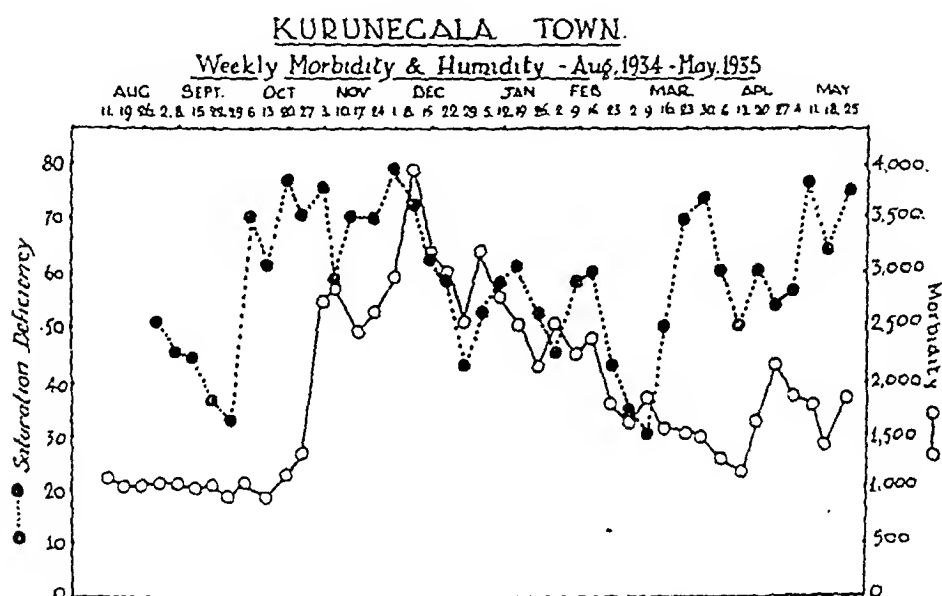
It will be seen that a sharp rise of atmospheric humidity occurred 3 weeks before the outbreak of the epidemic. It will likewise be seen that the second wave in April, 1935, was preceded by another sharp rise of atmospheric humidity about 1 month earlier.

It is conceivable that these sharp rises of atmospheric humidity may have

exercised an influence upon the host-parasite relationship whereby an "epidemic of relapses" was precipitated amongst human carriers. It is not possible to offer any suggestion regarding the nature of this influence or its mode of action, but the malaria parasite exhibits a quotidian, tertian, and quartan periodicity, which can be called into play by a wide variety of external stimuli, and it is conceivable that a sudden change of atmospheric conditions might in certain circumstances favour the occurrence of relapses amongst human carriers.

It is proposed, therefore, as a working hypothesis, to assume that a sharp rise of relative humidity during the pre-epidemic period is in some way respon-

FIG. 13.



Note.—(1) For the convenience of charting, the saturation deficiency figures have been subtracted from 100 in order to convert low figures (high atmospheric humidity) into high figures.

(2) The humidity figures refer to the mean weekly figures for the week *beginning* with the date shown in the chart, whilst the morbidity figures represent the figures for the week *ending* the stated date.

sible for the occurrence of a qualitative change in the malaria parasite—a change in the "epidemic potential"—at the commencement of epidemics, but it is not suggested that this rise of atmospheric humidity exercises any direct influence upon the malaria parasite, since it may only be the reflection of the influence of some other factor or factors with which it is closely correlated.

The only justification for the adoption of this hypothesis is the fact, which will now be demonstrated, that it appears to provide a means whereby many hitherto obscure features exhibited by malaria epidemics can be explained.

(A) SPATIAL DISTRIBUTION.

As the only available method of measuring the intensity of malaria epidemics is the mortality, mainly amongst children, to which they give rise,

it follows, other things being equal, that an epidemic of high intensity can only occur in an area where the proportion of children (non-immunes) is relatively high.

It likewise follows that there must be an adequate reservoir of infection, an abundance of good insect-carriers, and environmental conditions highly favourable to the transmission of infection.

The present hypothesis postulates that, given the above conditions, the spatial distribution of the epidemic and its focal character will be determined by the magnitude of the epidemic potential, and it is a remarkable and perhaps significant fact that the focal distribution of the Ceylon epidemic, as depicted in Fig. 4, corresponds closely with the excess over normal of the rise of relative humidity in October, 1934, the figures being Kurunegala, 8; Kandy, 6; Ratnapura, 9; Anuradhapura and Pattalum, 5; Trincomalee, 3; Colombo, and Galle, 4; Mannar, 1; and Jaffna, 0.

In the area of highest intensity—the central nucleus—not only was the epidemic potential higher than elsewhere, but the spleen-rate was moderately high, but not too high to occasion a low proportion of children or too low to be unfavourable to the occurrence of a widespread epidemic of relapses. On the other hand, the environmental conditions, in spite of the drought, were highly favourable to transmission, whilst the influence of the drought upon the river-beds created an environment conducive to the prevalence of the local insect-vector, but it is not thought that any special significance attaches to the preference of *A. culicifacies* for breeding in river-beds, since, as will be mentioned shortly, drought is associated with epidemics of malaria in tropical countries where this species does not occur.

To the south of the epidemic area—the districts of Galle, Matara, and Kalutara—the epidemic potential is normally extremely low (0 at Galle), and so also is the spleen-rate (0–5 per cent.), and hence in this area no appreciable epidemic would be expected to occur, in spite of the drought and famine, and in spite of the high proportion of non-immunes, even in the presence (which is, however, doubtful) of a sufficiency of insect-vectors.

On the other hand, in the hyper-endemic area the intensity of the epidemic would not be expected to be high, as measured by mortality, in spite of a high epidemic potential, owing to the small proportion of children and relatively high communal immunity.

The part ascribed to the epidemic potential also enables an explanation to be offered of the fact that malaria epidemics show a special liability to recur in approximately the same area.

In the epidemic area in Ceylon the epidemic potential is *normally* low, and much lower than in the hyper-endemic area, whilst economic conditions are ordinarily good. In these circumstances a natural recovery-rate of about 20 per cent. per annum might be expected—in the epidemic area in the Punjab the spleen-rate, which may have been 90 per cent. immediately after an epidemic,

returns to normal (about 5 per cent.) in about 5 years—and hence the “ epidemic status ” of the community in the epidemic area may be expected to return to its pre-epidemic condition in about 5 years.

On the other hand, in the hyper-endemic area, where the epidemic potential is normally high, and where the annual epidemic constitutes almost an annual epidemic, and where, moreover, economic stress constantly prevails, the natural recovery-rate would be expected to be small, with the result that the splcen-rate would become stabilized at a high figure (hyper-endemic malaria), and the epidemic status of the community would remain constantly in the condition characteristic of the post-epidemic period in an epidemic area.

(B) DROUGHT AND RAINFALL EPIDEMICS.

By postulating that a rise of atmospheric humidity during the pre-epidemic period is an essential precursor of an epidemic of malaria it becomes possible to explain the paradox associated with the fact that in Northern India excessive rainfall is an essential determining cause of malaria epidemics, whilst in Ceylon (Wet Zone) these epidemics invariably occur in association with drought.

In the latter area, as already stated, the atmospheric humidity remains relatively constant at a high level throughout the year, with the exception of a small rise in June in association with the onset of the south-west monsoon and another small rise in October following the onset of the north-east monsoon.

It thus comes about that a deficiency of rainfall during the first 5 months of the year occasions a progressive decline of atmospheric humidity during the first 5 months of the year, which is followed, as the result of the onset of the south-west monsoon, by a sharp rise of atmospheric humidity in June. This sequence of events occurred in the Wet Zone of Ceylon in the years 1906 and 1911, when a sharp rise of atmospheric humidity in June was followed by the well-marked epidemic waves shown in Figs. 8 and 9.

On the other hand, when the rainfall during the first 5 months of the year is excessive, no progressive decline of atmospheric humidity occurs, and, in consequence, the onset of the south-west monsoon is not followed by any appreciable rise of atmospheric humidity in June. This sequence of events occurred in the year 1934, and is illustrated in Fig. 14 (p. 458), from a scrutiny of which it will be seen that the normal summer wave is missing.

Similarly, a failure of the south-west monsoon in the Wet Zone of Ceylon occasions a progressive decline of atmospheric humidity during the months of July, August and September, with the result that the rainfall in October, although it may be below normal, occasions a large rise of atmospheric humidity.

This was the sequence of events during the latter half of the year 1934, when the prolonged drought during the months of July, August and September was followed by an abnormally large rise of atmospheric humidity in October (*vide* Fig. 13) and by the outbreak of the epidemic at the end of the month.

It is thus clear that in Ceylon (Wet Zone) the main circumstance responsible for a large rise of atmospheric humidity in June and October is the preceding drought, and hence it is permissible to speak of such epidemics as drought epidemics. It is likewise clear that the significant factor is not the percentage of atmospheric humidity prevailing during the epidemic but the rise which it undergoes during the pre-epidemic period, and on this basis it is possible to understand why the greatest drought on record should have been followed by the greatest malaria epidemic in the history of Ceylon. Finally, it is possible

FIG. 14.

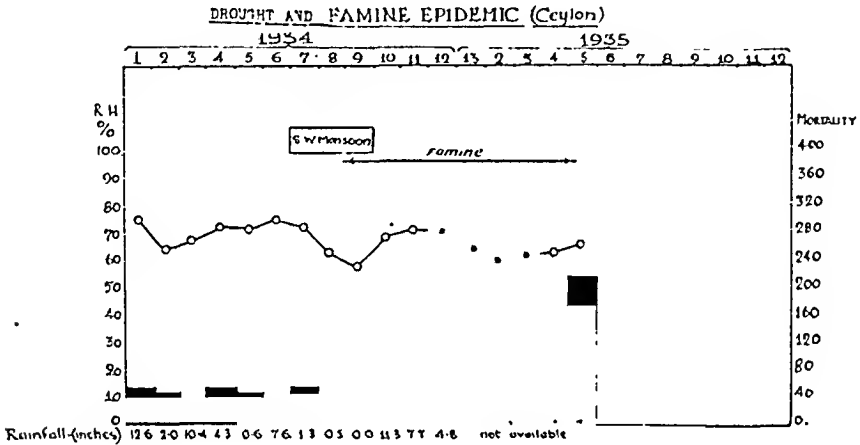
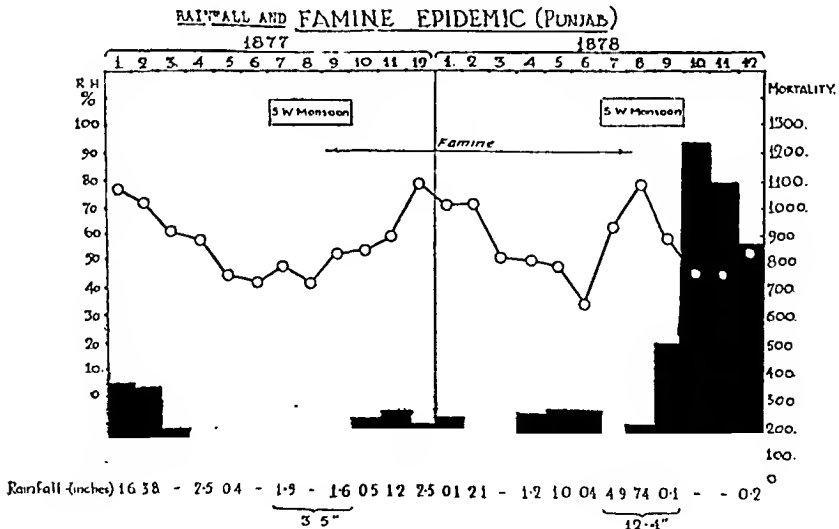


FIG. 15.



to explain why the first wave of the Ceylon epidemic occurred in the winter and not in the summer of the year 1934, and why in the years 1906 and 1911 the first wave occurred in the summer.

The monthly atmospheric humidity prevailing in an area where malaria epidemics are dependent upon excessive monsoon rainfall is illustrated in Fig. 15, from a scrutiny of which it will be seen that in the Punjab the atmospheric humidity, except during the winter, is extremely low until the month of July when it undergoes a rise proportionate to the amount of the monsoon rainfall. In the year 1877, owing to the complete failure of the monsoon, the usual rise of relative humidity in July and August did not occur, and in association therewith, there was a complete absence of epidemic malaria in the autumn; indeed, in spite of famine, the public health remained exceptionally good. On the other hand, in the following year, the abnormally heavy monsoon rainfall in July and August was associated with an extremely large rise of relative humidity (from 38 per cent. in June to 79 per cent. in August) and by an exceptionally severe epidemic of malaria during the last 4 months of the year.

Here therefore the excessive rainfall during July and August was the main factor responsible for the large rise of atmospheric humidity during the pre-epidemic period, and it is justifiable to regard this type of epidemic as a rainfall epidemic, and to expect a high degree of correlation between the July-August rainfall and the autumnal mortality from malaria.

The essential features of drought and rainfall epidemics are displayed diagrammatically in Fig. 16. It is inferred that a drought epidemic can only occur in areas where the atmospheric humidity is normally high and where a drought occasions a temporary fall of atmospheric humidity. Such an epidemic may therefore be described as a V-type epidemic, in which the right limb of the V represents the epidemic potential, whilst a rainfall epidemic may be termed an inverted V-type epidemic, the left limb of the inverted V representing the epidemic potential.

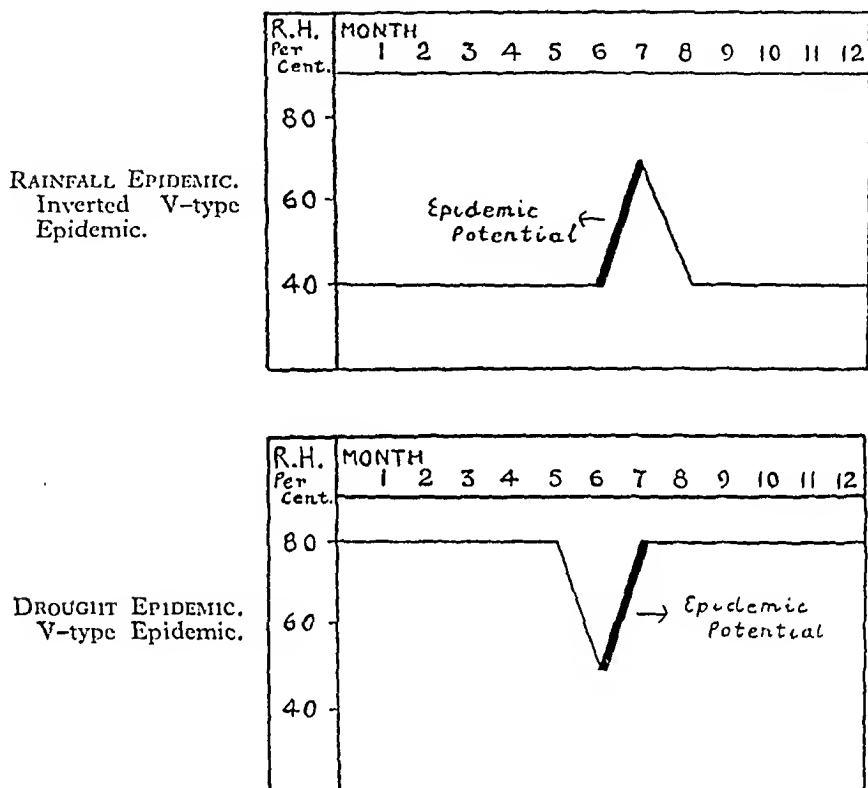
It is however clear that in both types of epidemics the sequence of events is the same, since a sharp rise of relative humidity is common to both, although in the one case it is rendered possible by preceding drought and in the other by excessive rainfall.

In conformity with this explanation of the mechanism of drought and rainfall epidemics it is found that drought epidemics occur in those parts of Bengal, Assam, Bombay, Ceylon, Malaya, Mauritius and British East Africa, where the atmospheric humidity is normally high throughout the year, whilst rainfall epidemics occur in those parts of these and other countries in the tropical and sub-tropical zones where the rainfall and atmospheric humidity is normally low (excluding the cold season) except during the rainy season.

In the temperate zone the atmospheric humidity is high throughout the year and malaria epidemics appear to be drought epidemics, and, since a dry summer in northern and central Europe implies a hot summer (which is also

favourable to the anopheline factor) a positive correlation exists between " malaria " and " temperature " in this zone.

FIG. 16.



The above interpretation of the mechanism of drought and rainfall epidemics may or may not be correct in whole or in part, but the conclusion appears to be justifiable that the occurrence during pre-epidemic periods of droughts in certain parts of the world and excessive rainfall in others is capable of determining the occurrence of malaria epidemics.

(c) CYCLICAL PERIODICITY.

In view of the part attributed to droughts and floods in determining epidemics of malaria, it follows that meteorological cycles associated with the occurrence of droughts and floods at periodic intervals would occasion a similar periodicity of malaria epidemics. A tendency for these epidemics to occur at more or less regular intervals has frequently been noted. Thus CELLI (1908, 1925), who appears to have been the first to remark on this character of malaria

epidemics, states that since the middle of the 19th century epidemics have occurred in the Roman Campagna with approximately a 10 year frequency whilst intermediate 5 yearly fluctuations are also evident. GILL (1928) states that in the Punjab major epidemics of malaria often occur at intervals of approximately 10 years and that minor epidemics sometimes occur midway between the major ones. YOUNG and MAJID (1930) state that in Northern Sind malaria epidemics occur at approximately 10 yearly intervals, major epidemics being recorded in the years 1897, 1906, 1917 and 1929.

BARBIERI (1931) states that in the Argentine, Parana, and Uruguay malaria epidemics occur every 11 or 12 years, and ET. SERGENT (1932) states that epidemics have occurred in Algeria every 12 years since the year 1902. In Ceylon malaria epidemics have occurred at intervals of approximately 5 years since the year 1867, whilst a similar tendency to a 5 yearly periodicity is discernible in the mortality statistics of Mauritius since the year 1867.

Little has been heard of pandemics of malaria during the present century but HIRSCH (1883) records many great outbreaks of malaria in the 19th century in which he states "attain a wide diffusion over great tracts of country and whole regions of the globe and run their course, not within one season or even within one year, but often last for several years and then remain absent for years or even tens of years."

He states that malaria was pandemic in Europe in 1806, that a great epidemic occurred in South India and Mesopotamia in 1811, that about the year 1820 an extensive, severe and persistent pandemic prevailed in almost all parts of the world, that malaria exhibited unwonted prevalence in many countries in the years 1845-1849, 1855-1860 and 1867-1872.

The last mentioned pandemic involved many countries in Europe, North and South America and several countries in Asia, including India and Ceylon whilst in the years 1867-1868 Mauritius was decimated by the greatest malaria epidemic on record. The years 1877-1879 were associated with malaria epidemics in many parts of Europe and Asia (India, Ceylon and Mauritius) and malaria was again epidemic in India, Malaya, Ceylon, and Mauritius in the years 1900-1902.

It seems therefore permissible to speak of malaria epochs, and it is clear from a scrutiny of the above figures, that these epochs exhibit a tendency to 10 yearly periodicity, thus confirming the observation of CELLI in respect of Italy.

CELLI (1925) attributed the periodicity of malaria epidemics to the waxing and waning of the inherent periodicity of the malaria parasite, which, he states, occurs independently of climatic and meteorological conditions. "Malaria," he remarks, "like all other epidemic diseases is a periodic phenomenon. It develops in waves, cycles, and rhythms, which recur with periodic frequency daily, monthly, and annually," and he also distinguished, as the result of a

exhaustive study of the malarial history of the Roman Campagna, a grand cyclical development during the centuries.

GILL (1928) suggested, in view of the tendency of malaria epidemics to occur in the Punjab at intervals of about 10 years, that this periodicity might be related to the 11 year sun-spot cycle; and BARBIERI (1931) and ET. SERGENT (1932, 1933) state the malaria epidemics in South America and Algeria respectively are to some extent associated with certain phases of the sun-spot cycle.

The first person to associate the sun-spot cycle with malaria epidemics was C. MELDRUM, LL.D., F.R.S. (1881), Director, Meteorological Observatory, Mauritius, who after making an exhaustive study of the meteorological conditions prevailing in Mauritius at the time of the great epidemic in the year 1867, came to the conclusion that one of the factors responsible for this epidemic was the abnormal meteorological conditions prevailing not only in Mauritius but widely throughout the world at this epoch, and, although handicapped by the paucity of data regarding other countries, he definitely attributed the high mortality that occurred at this epoch in Mauritius, India, Ceylon, and certain other countries to the abnormal world-weather associated with the commencement of the sun-spot cycle in the year 1867.

He stated—and this view is generally held by meteorologists at the present day—that at the turning points representing the inequality of solar activity, as represented by the comparative presence or absence of spots, *faculae*, and prominences on the sun, the weather is subject to great extremes, with the result that floods and droughts are particularly liable to occur at the period of maximum and minimum sun-spot numbers, and more especially at the epoch of minimum sun-spot numbers.

The sun-spot cycle is however of variable duration, the period from one minimum to the next varying between 10–12 years, the average being 11·3 years. It also exhibits another inequality since the rise from the minimum to the maximum is usually more rapid than the decline from the maximum to the minimum, so that the epoch of the maximum, instead of occurring about $5\frac{1}{2}$ years after the minimum, is usually reached in about 4 years, whilst the decline from the maximum to the minimum usually takes about 7 years.

In these circumstances it is necessary, in order to determine the relationship between the sun-spot cycle and the periodicity of malaria epidemics, to examine the annual sun-spot numbers, and accordingly in Fig. 17, a curve is shown based upon the sun-spot numbers during the period 1810–1934.

The most striking feature of this curve is the remarkable association of great malaria epidemics and pandemics with the epoch of minimum sun-spot numbers. It will be seen that all the pandemics mentioned by HIRSCH between the years 1810 to 1867 coincide with a period of low sun-spot numbers. Furthermore, HIRSCH records the occurrence of a pandemic at every epoch of minimum sun-spot numbers during the above period, with the exception of the years 1833–1834.

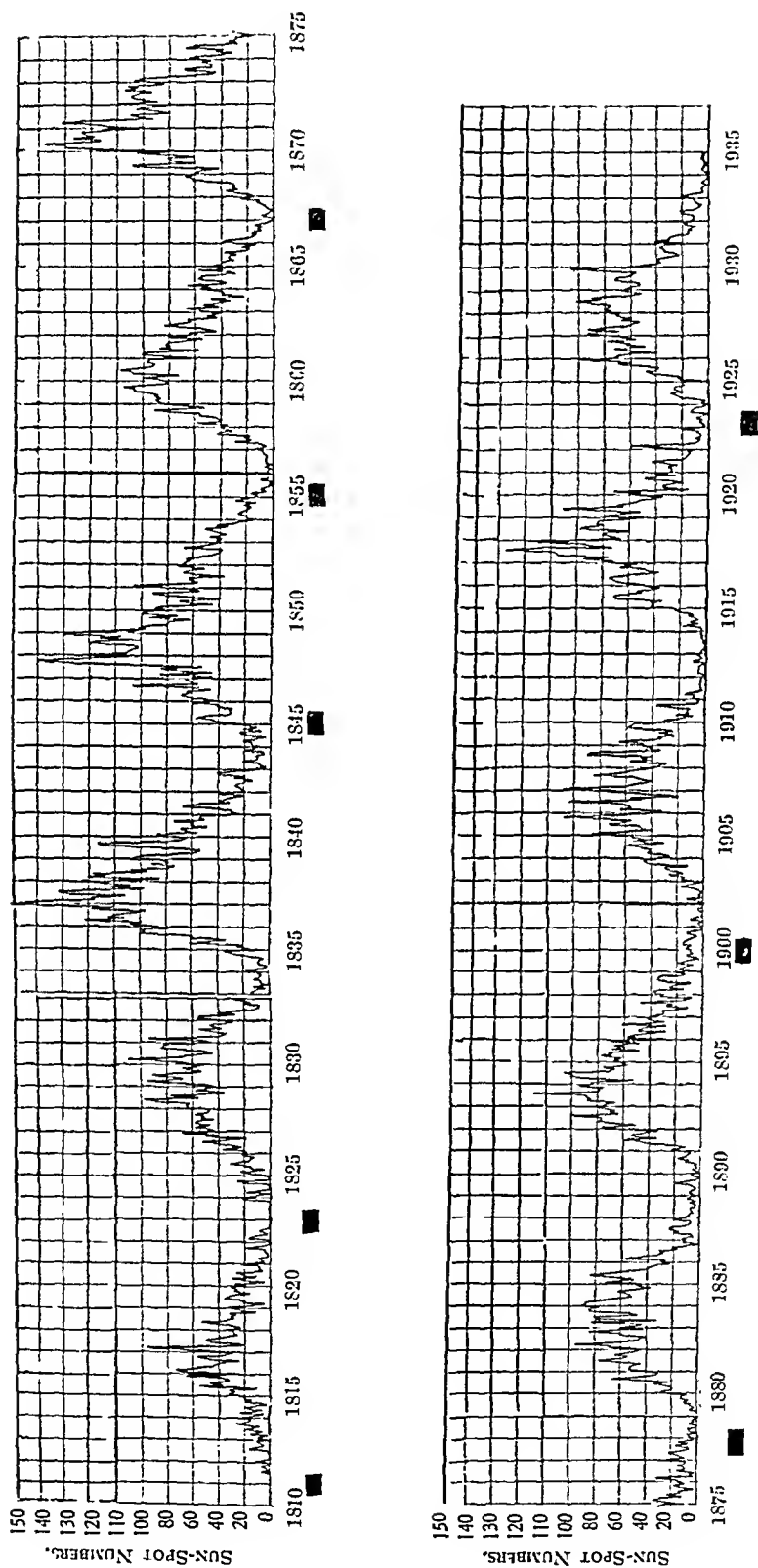


FIG. 17.— CURVE OF SUN-SPOT NUMBERS, 1810-1934.

Malaria pandemics mentioned by Hirsch and the pandemics of 1878, 1900 and 1923 are indicated by ■.

(Chart kindly supplied by Dr. G. M. FINDLAY.)

The next pandemic, after the great pandemic of 1867-1869, occurred at the period of minimum sun-spot numbers in 1877-1878. There was no pandemic in the years 1889-1890, but epidemics are recorded in Northern India, Mauritius, and Ceylon in these years. The period of minimum sun-spot numbers in 1900-1901 was associated with a great famine epidemic in the Punjab, and with malaria epidemics in Malaya and Mauritius.

The period of minimum sun-spot numbers in 1911-1912 was not associated with a great pandemic, but malaria epidemics occurred in Ceylon, Malaya, and Mauritius.

The epoch of low sun-spot numbers in the year 1923 was associated with a great famine epidemic in Russia, in which the Balkans were also involved. Minor epidemics occurred during this year in the Punjab and in Ceylon.

Finally, the recent epidemic in Ceylon occurred in association with the new sun-spot cycle commencing in the year 1934. The sun-spot number for the year 1935 (up to September) is 28.5, and it may therefore well be, if the present abnormal world-weather continues, that epidemics of malaria will be reported from other countries before the close of the current year.

Scarcely less striking is the association of malaria epidemics with the epoch of maximum sun-spot numbers. Thus in the Punjab major epidemics occurred in the years 1861, 1870, 1884, 1892, 1894, 1908 (which is however somewhat later than the year of maximum sun-spots), and 1917.

No epidemic took place in the Punjab in the year 1928, but a major epidemic occurred the following year in the adjoining province of Sind.

In Ceylon, the association of malaria epidemics with the epoch of maximum and minimum sun-spots is extremely close. Thus, epidemics of varying magnitude took place in the years 1906, 1911, 1914, 1919 (?), 1923, 1928, and 1934, all of which, with the exception of the doubtful epidemic in the year 1919, occurred about the epoch of maximum or minimum sun-spot numbers. There is thus a considerable amount of evidence pointing to the conclusion that a definite relationship exists between the sun-spot cycle and the periodicity of malaria epidemics. Nor is there anything obscure about this relationship, since if the meteorologists be correct in stating that a special liability exists for droughts and floods to occur at the epoch of maximum and minimum sun-spots, it would be expected that malaria epidemics would exhibit a tendency to occur at intervals of 5 and more especially of 10 years approximately.

The oscillations of solar activity, of which sun-spot cycle is one manifestation, are also associated with magnetic disturbances, and it is conceivable therefore that variations in the quality or quantity of solar radiation at the time of maximum and minimum sun-spot numbers may be the precipitating cause of the outbreak of relapses at the commencement of malaria epidemics.

For the present it will suffice to state that the relationship of malaria

epidemics to the sun-spot cycle is sufficiently close to permit of the conclusion that the cyclical periodicity of malaria epidemics is attributable to the changes in world-weather associated with periodic oscillations in solar activity.

6.—CONCLUSIONS.

The conclusions reached as the result of the study of the malaria epidemic in Ceylon may be briefly summarized as follows :—

(1) It is held that the epidemic was initiated by an " epidemic of relapses " occasioned by a change in the parasite-host relationship.

(2) The general determining cause of the " epidemic of relapses " is unknown, but it is inferred that it is closely related to or correlated with an abrupt rise of atmospheric humidity during the pre-epidemic period.

(3) The adoption of the above hypothesis renders it possible to explain many peculiar features exhibited by malaria epidemics, more especially their sudden and almost simultaneous onset over wide areas, their spatial distribution and focal character, their wave form, their association with drought in certain countries and with excessive rainfall in others, and their seasonal and cyclical periodicity.

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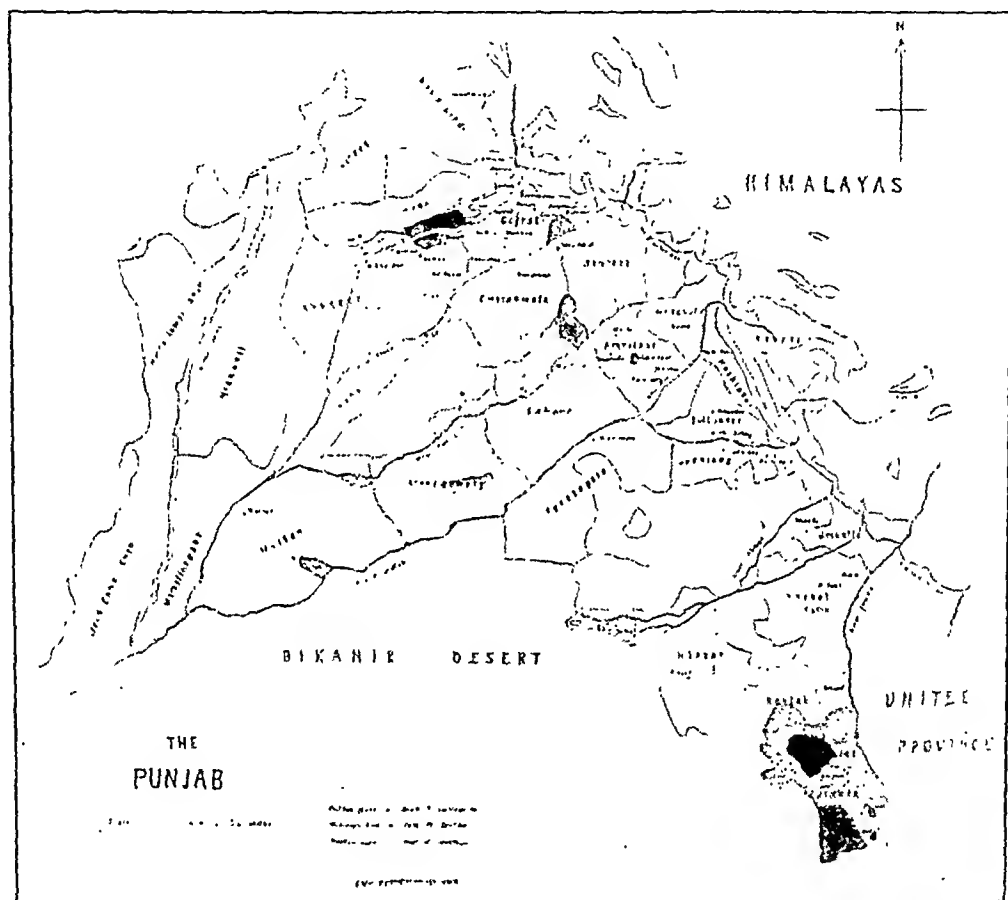
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DISCUSSION.

Sir Rickard Christophers : Any considerable enhancement of malaria in a community may be termed an "epidemic." There are, however, many types of such "epidemics" differing in their mechanism of origin, in their characters and in the scale on which they are manifested. Small "outbreaks" are very commonly described in the literature. In these the epidemiology is frequently very simple. A community of non-immunes is exposed to conditions very suitable for malaria transmission, as for example to a considerable degree of anophelism, infection is rapidly disseminated and the whole community goes down with malaria. Larger epidemics usually have a more complex epidemiology and it would appear that "immunity" is usually a large regulating factor. A form of epidemic which may occur on a considerable scale is that known as the "malaria of tropical aggregation of labour." Among a number of factors here operative the protective rôle of immunity is ruled out through the continued influx of non-immunes bringing about a condition which may be likened to the heaping of fuel upon a fire. A not uncommon condition in India is the increase of malaria in a tract over a period of years so that this tract, originally healthy, suffers from what practically amounts to a long drawn out epidemic. The type of epidemic of greatest importance and about which most is known in India is, however, that termed "fulminant autumnal epidemic malaria" or "regional epidemic malaria" as it is now generally described. This has very definite characteristics among which may be noted (1) a marked seasonal relationship so that its dates of onset and duration may be almost identical in different years, (2) an extremely sudden onset, (3) synchronicity of onset over very wide areas amounting often to hundreds of square miles, (4) a marked focal distribution of intensity and, (5) a very high associated mortality. Though the Northern Indian epidemics are apparently related to "flooding" and the Ceylon epidemic to "drought" the similarity in other respects is so striking that one may safely place the Ceylon epidemic in this class. The maps show two of the North Indian epidemics prepared by the same method as was adopted by Colonel GILL in mapping the Ceylon

epidemic, *viz.*, by use of the epidemic figure. These are the epidemics of 1908 and 1892 respectively. The maps were published in my paper many years ago on Malaria in the Punjab.* It is probable, however, that very few here have seen them. The area shown is that portion of North-West India lying between the Himalayas and the mountains of Baluchistan and forming the great plains of the Punjab. The whole of Ceylon would fit comfortably into the corner occupied by the northern epidemic in 1908. This year was unique in that there were two simultaneous but distinct epidemics. The



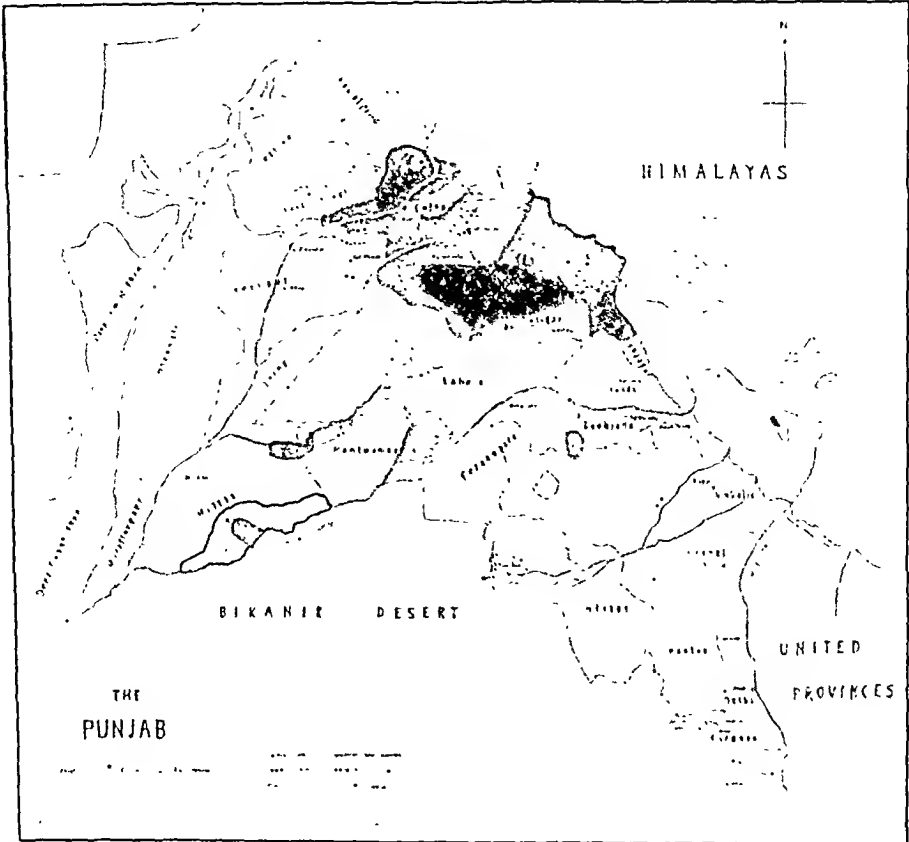
MAP 1.—Map of the Punjab showing distribution of the malaria epidemic of 1908 (two simultaneous epidemic areas). Scale 1 inch = 96 miles. Light shading = epidemic figures of 5 or over. Medium shading = epidemic figures of 7 or over. Dark shading = epidemic figures of 10 or over.

epidemic of 1892 is almost certainly the greatest recorded single epidemic of this type there has ever been in the world. The extra mortality due to the epidemic in 2 months being of the order of 150,000 deaths.

No one looking at these maps, or that prepared by Colonel GILL of the Ceylon epidemic, can fail, I think, to be struck by the resemblance to maps of meteorological phenomena. They are, one feels instinctively, maps of

*CHRISTOPHERS, S. R. (1911). Malaria in the Punjab. *Sci. Mem. Off. Med. San. Dept. Gov. India* (New Series) No. 46. Calcutta: Superintendent Government Printing, India.

"malaria eyclones." This resemblance is not merely superficial, but is due to the fact that the fundamental causation of these epidemics is meteorological. The immediate meteorological happenings of the year in which the epidemic occurs can be readily shown to be concerned, by the comparison of rainfall maps etc., with the epidemic distribution. This, however, is not the whole story since the succession of meteorological events throughout the previous decade or more obviously also have their effect. One may say that there is the sensitizing and exposure of the plate, as well as the developing, to be considered. That there



MAP 2.—Map of the Punjab showing distribution of the malaria epidemic of 1892. Scale 1 inch = 96 miles. Light shading = epidemic figures of 5 or over. Medium shading = epidemic figures of 7 or over. Dark shading = epidemic figures of 10 or over.

should be evidence in the occurrence of such epidemics of "cycles" and even of a relation to sun-spots is more than probable once one recognises their dependence on meteorological events. Unfortunately whilst this indicates to us the general nature of the fundamental causes, it fails to tell us very much as to the mechanism by which the results are brought about, the details of which as epidemiologists we above all wish to know.

Time does not permit of a discussion of many points raised by Colonel GILL. The most interesting and important part of Colonel GILL's theory

concerning the mechanism of the epidemic appears to me to be his observation that the first wave, *i.e.*, the outburst of cases starting almost everywhere about the 27th October, was unassociated with the mortality one might have expected among the young children. It is startling to think that this sudden outburst could be due solely or mainly to relapses, especially since it is difficult to assign any apparently adequate cause likely simultaneously to precipitate so many relapses. But the author's arguments are not easy to controvert and his contention must, I think, be given serious consideration. I should like to ask Colonel GILL if there was any collateral evidence obtainable from the dispensaries that these early cases were of a milder character than the later ones as one might expect if they were relapses. On the other hand evidence of such mildness does not necessarily prove the relapse theory since the mildness of the cases in the beginning might be a result of numerically poor infections in the anophelines.

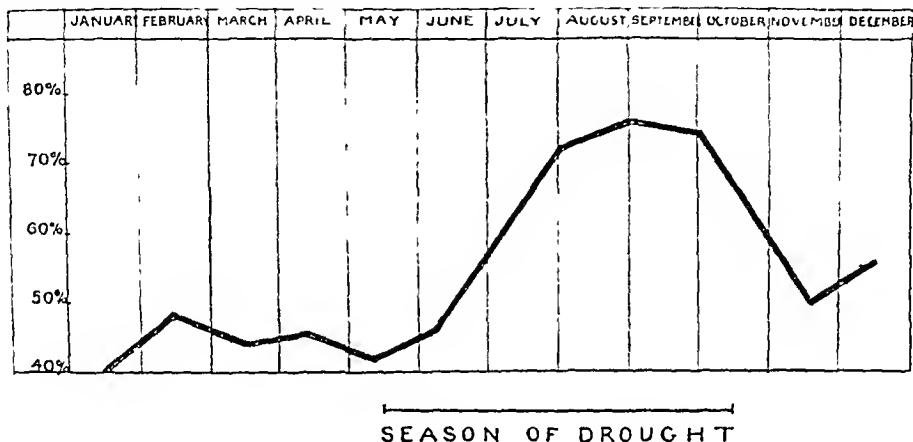
Colonel S. P. James : As Colonel GILL was so kind as to mention the prediction I made to the Colonial Office that there would be a second wave in the spring, I had hoped to show to-night some lantern slides to explain the reasons on which that prediction was based ; but, in view of the short time available for discussion, I will leave out that part of what I wanted to say and will refer only to the causation of the primary wave.

This primary wave is very puzzling, and the explanation which Colonel GILL has given of it to-night is new and unexpected. If I understood him correctly, he regards the drought as the prime and essential cause, but attaches little or no importance to the phenomenal increase of *Anopheles culicifacies* which accompanied it. Instead of this anopheles abundance I understand that he attaches most importance to the occurrence of an epidemic of relapses preceding the epidemic wave.

The first factor is the drought. This, like every other factor in epidemiology, acts in different ways in different countries. The simplest, and one of the clearest examples of the influence of drought is seen on the north coast of Java, where an epidemic occurs annually during the dry season. As Dr. DE VOGEL* discovered many years ago, it happens at that time of the year that freshwater pools along that coast partially dry up and become disconnected from the canals which normally supply them with larva-eating fish, and the water becomes so hot that it kills the fish, but the mosquito larvae thrive and multiply enormously. The graph on page 470 shows that this epidemic during the dry season in Java begins a few weeks after the onset of those conditions, and attains its maximum in August and September, which is the middle of the dry season. It is obviously an epidemic due to drought. Evidently the drought in Ceylon did not act in that way. It began after the drought had ceased, and then it began suddenly, so suddenly, as Colonel GILL says, that its onset over nearly the whole area could be fixed to a day. This sudden onset must be accounted

* DE VOGEL (1907). *Atti d. Soc. per gli Studi d. Malaria*, viii.

for in any explanation of the epidemic, as also must his description of it as being explosive in nature.* The explanation must also show us why no progressively increasing abundance of anopheles occurred between June and September, while the rivers were drying up. Also the explanation must accord with Colonel GILL's view that the drought was the prime cause of the epidemic. I think Colonel GILL envisages only one explanation which fulfils all those requirements, namely, the explanation that prior to the sudden explosive onset on 29th October, an equally sudden and explosive onset of relapses occurred among human malaria carriers.



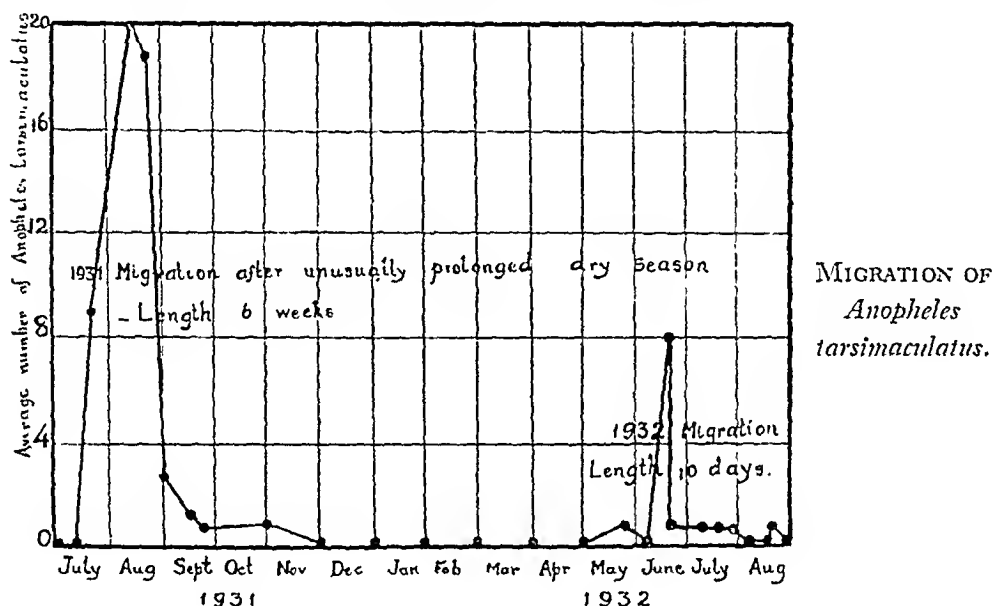
ANNUAL MALARIA EPIDEMIC. NORTH COAST OF JAVA.

I had hoped to develop alternative explanations in my remarks this evening, but, as I have not time to do so, I will only bring to notice a suggestion which was made to me by Dr. DE VERTEUIL, of Trinidad. It is that this sudden explosive epidemic was caused by a sudden invasion of anopheles from somewhere outside the particular epidemic area in which the onset was explosive; that is, that at the first onset of rain after the drought there occurred one of those strange migrations or flights of anopheles which happen from time to time at certain seasons in different countries. A long time ago I recorded a migration of this kind in my report on the antimalarial operations at Mian Mir in 1902, but at that time no one was willing to believe that anopheles could fly more than two or three hundred yards. Ten years later, however, the same phenomenon was reported from the Panama Canal, and then workers began to sit up and take notice. Between 1924 and recent years KLIGLER studied the subject thoroughly in Palestine. Most important of all for our present purposes are the observations of Dr. DE VERTEUIL himself, for he has kept exact records of some exceptional migrations of anopheles in Trinidad. He finds that migration on a small scale occurs annually; but that, at intervals, and always after a prolonged

* I should like it to be understood that my remarks are applicable only if the epidemic did, in fact, begin suddenly and explosively. If it began gradually its explanation is less difficult.—S. P. J.

drought, migration occurs on an immense scale. The graph below is from Dr. DE VERTEUIL's report* of a migration which followed a prolonged drought in 1931. It was a migration which lasted 6 weeks, as compared with only 10 days in 1932.

Of course, this hypothesis of migration does not necessarily upset Colonel GILL's view of an epidemic of relapses; it may supplement rather than supplant it. His statement that the primary wave began on the same day over a large area seems to indicate a sudden invasion by *infected* anopheles—in which case it would not be necessary to suppose that there was a pre-epidemic wave of relapses. KLIGLER, in his investigations in Palestine, reported obtaining



"absolute proof" of the long-range dispersal of infected anopheles from infected to uninfected villages several kilometres distant.† If, however, the migratory swarm did not include infected insects one has to postulate a coincident wave of relapses to account for the simultaneous infection of large numbers of anopheles and the resulting explosive outburst of clinical cases. However, if there was this wave of relapses I should have expected that the statistics of the year would have shown a corresponding wave of primary attacks about 8 months previously, namely, in February. I should like to add that if Colonel GILL could show us temperature charts of the very earliest cases in the epidemic, I think we could tell him whether they were primary attacks or relapses.

I must not speak longer, so I will only say that it seems impossible to decide,

* DE VERTEUIL, E. (1933). Malaria survey for 1932, *Trinidad and Tobago Council Paper*, No. 32.

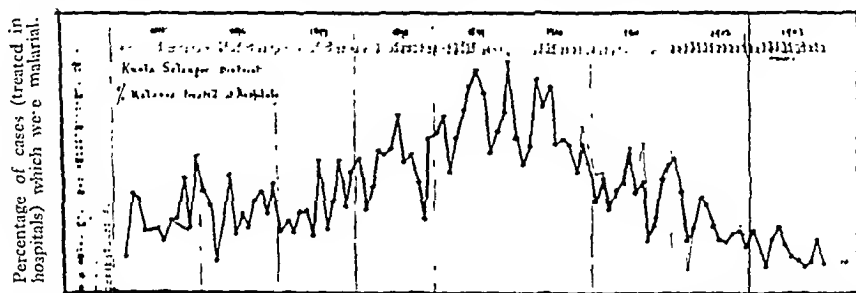
† See for example: KLIGLER, I. J. (1932). The movements of anopheles at various seasons of the year with special reference to infected mosquitoes. *Trans. Roy. Soc. Trop. Med. & Hyd.*, xxvi, 73.

until continuous work has been done over a series of years, whether this epidemic was due to a sudden invasion of infected or uninfected anopheles, or to a pre-epidemic wave of relapses, or to other factors which are, as yet, unknown. I have no strong feelings on the subject, except to urge that further research should be carried out. It is unfortunate that, although *culicifacies* was proved to be an important carrier of malaria in India more than 30 years ago and in Ceylon more than 20 years ago, we have as yet no moderately complete account of the life-history of this insect in its adult stage. I stress this because I have always held that for the purposes of malarial epidemiology and control it is more profitable to study the life history and habits of the insect vector in its adult stage than it is to study the breeding places of its larvae.

Sir Malcolm Watson : The predominant feeling of everyone in this Society is, I am sure, deep sympathy with the people of Ceylon in the misery and distress, sickness and death which have afflicted them in the past year. It is urgent that Ceylon should get every possible help because epidemics on a larger and smaller scale have occurred in the past and there is no reason to doubt that they will recur in the future, unless some means are taken to control them.

In his paper to-night, Colonel GILL quite frankly states his opinion that, except for small "closed" populations, there is no hope of controlling malaria; and that for "the large free populations of rural areas" . . . "the control of malaria as a practical proposition is almost as far to seek as it was a century ago."

I can hardly believe that this Society will allow any such statement to pass unchallenged: I certainly am not prepared to do so. On the other hand, I do not propose to spend much time in directly controverting Colonel GILL's views, I propose rather to speak on a constructive policy but wish first to emphasize two points. The first (see chart below) concerns a wave of malaria among a free population in a wide rural area which was studied over 30 years ago. That area was occupied by Malays, Javanese, Banjarese, Chinese and other Asiatics living their own lives on their own small holdings much as the



The Chart (here published for the first time) shows the effect of agricultural drainage in controlling a severe and increasing outbreak of malaria in the District of Kuala Selangor, F.M.S. The figures on which the Chart is based were published in my *Prevention of Malaria in the F.M.S.* The drainage was begun in 1898.

people of Ceylon do to-day. The only European estates were three semi-derelict and practically bankrupt coffee estates with a total acreage of about 600 acres. A study of that epidemic showed us the cause of the rise and the fall of the epidemic. It was the starting point of the control in Malaya of rural malaria under various conditions and by diverse methods dependent on the species of mosquito which carried the disease. The second point is that the malaria course for laymen held at the London School of Hygiene and Tropical Medicine shows an ever increasing number of attendances. Would laymen attend that course if nothing more practical was to come of it than Colonel GILL seems to suggest?

The people of Ceylon are probably now realising, as they have never realised before, the significance of the high spleen-rates which have been known to exist in several areas of the island for at least the past 25 years. It will be easy to waste money, and money will certainly be wasted, unless the prevention of malaria is preceded by co-ordinated research on a much larger scale than has hitherto taken place in Ceylon. I have had the pleasure of seeing some of Mr. H. F. CARTER's excellent work in his own company. I have nothing but praise for it; but I am sure that Mr. CARTER himself would agree that had there been more money and greater facilities provided, the quantity of both the research and practical work which he has done would have been much greater.

On receipt of Colonel GILL's report, it struck me, as it struck several others who read it, that it was not as helpful from the practical point of view as it might have been. Accordingly, I wrote a memorandum in which I have discussed the Ceylon epidemic in the light of my experience of malaria in many countries and particularly in Malaya which, like Ceylon, is situated in the equatorial zone and has many other similarities. I have also discussed briefly the question of hookworm, oedema—which forms a prominent feature clinically in Ceylon, as it did in Malaya—housing, water supplies, site selection—even for the smallest social units—antimalarial drugs and hospitals.

On each of these subjects more local research is required in Ceylon if money is not to be wasted. It is of vital importance, for example, to determine the cause of the oedema which was so prominent a feature in the hospitals. Is it due to malaria or to the hookworm, or both? The work of STANTON, MACAULAY and myself in Malaya gave us a definite answer; and our conclusions were confirmed by the Rockefeller Hookworm Commission to the Orient, 1915-17. The Commission's Report has an important paragraph (page 110) on "Severe malaria (cachexia) mistaken for hookworm disease." We want to know if the oedema in Ceylon is the same condition as it was in Malaya. It is useless to speculate; we require definite observations in hospitals, laboratories and in the field, and the work must be co-ordinated if a conclusive answer is to be given.

Another question: Why did the south of Ceylon escape the recent malaria epidemic? There are wells and rivers in the south as well as in the centre

and north of the island. Here again we want exact evidence. I would like to emphasize in connection with this work the value of the small field laboratories, organized by Dr. G. C. RAMSAY, in Assam, Bengal and Southern India. Work carried out in that way would be of the greatest value in Ceylon.

It is not possible to give in detail all I have written in my memorandum on the malaria in Ceylon, which has been in Colonel GILL's hands for some weeks and which I have discussed with him ; but the following are the two concluding sections. They indicate the basis of the malaria policy in Malaya and suggest a policy for Ceylon.

" The control of malaria was the foremost problem. That no satisfactory return would be obtained from expenditure on other sanitary measures as long as the people were sodden with malaria. That the site of the house was of vital importance, for a good site might eliminate malaria completely and at no cost, or where complete elimination was not possible, the right site reduced the cost of control to a minimum. Much of my work in my later years in Malaya was of this nature. Once a satisfactory site has been selected, then we can proceed with expenditure of money on other things, such as houses, water supplies, etc., but expenditure on these things before making sure that the site is satisfactory may be an obstacle to the control of malaria.

" I would also like to add that in Malaya, tuberculosis was a serious problem in the brick houses of towns. And its occasional presence in tightly sealed houses in villages led me to advocate houses on estates with wooden walls which, through warping of the planks, admitted free currents of air at all times—currents of air which the inhabitants could not easily obstruct.

" In countries like Malaya and Ceylon, where cholera is a menace, pure water is essential. The great majority of the estates in Malaya which came under my control were in time provided with supplies of good water, but these were not provided until we were satisfied that the site for the house was satisfactory and that it would be a permanent site. Adequate water supply permits of septic tanks and water-flushed latrines and on many estates these were provided ; bore-hole latrines came later.

" *Policy for Ceylon.*—Before large sums of money are spent on antimalarial work in Ceylon, a clear answer is required to the questions which I have discussed. Are the conclusions which I reached in Malaya true of Ceylon ? For if the conditions are the same in Ceylon, then the same general policy should be adopted as in Malaya ; if in Ceylon, the conditions are different, then a different policy will be necessary. But clear proof would be required that the conditions are different, especially in view of the recent great epidemic, which indicates that malaria is the major disease problem in Ceylon, as it was in Malaya ; and that being so the presumption is that the policy which has been, and is being, followed in Malaya is that best suited to Ceylon.

" And I would urge that the research I have outlined briefly should include the two-thirds of Ceylon which is now submerged in jungle ; I believe that research will show a way to reclaim this land.

“Formidable as the problem may appear, few I think, will regard it as more formidable than that presented by the malaria of the great swamps of Malaya, described in the Finlayson Lecture, from which I have already quoted. To-day we have complete knowledge of how to control that malaria; indeed in some places the control is so complete that Dr. BARROWMAN, my successor, wrote of Carey Island Estate as follows: ‘There has been one child with enlarged spleen during the past 5 years, and she had arrived on the Estate with the spleen already enlarged.’ . . . ‘There are over 1,500 healthy, happy children among whom the sick day rate last year was no more than 0·4 per cent.’ . . . ‘Carey Island is situated in the delta of the Klang and Langat Rivers. The land is low-lying soil with high ground water, in fact, it is naturally a fresh-water swamp surrounded by salt-water swamp, conditions in the tropics which are notorious for producing the most appalling malaria.’ . . . ‘This combination of measures on Carey Island is so successful and so unobtrusive that the whole population goes about its daily work doing nothing it would not do elsewhere, and unconscious that but for the control in force the island would be one of the death traps of the tropics.’

“That shows what research has done for one of the intensely malarial zones of land in Malaya; in other zones the results of appropriate measures have been not less striking. These results would in themselves be sufficient to encourage an attack on the jungle zone of Ceylon. But there is another reason. In that jungle zone are the ruins of cities, and the even more striking remains of a great irrigation system; obviously the work of a cultured and civilised people; most improbably the work of a race habituated to malaria. In the 12th century A.D., they were driven from their lands; yet when the wars ceased they were never able to return to them or recreate their former prosperity. Every attempt to do so has ended in disaster. Had intense malaria been present before and during the twenty centuries of their ancient occupation they would have acquired such a tolerance to the disease, that they could have returned without difficulty. If, as I believe, the land and people were free from malaria these disasters point our great hope.”

Professor D. B. Blacklock: Colonel GILL in his valuable contribution to the epidemiology of malaria stresses the importance of two factors; the first concerns the inherent periodicity of the simple tertian parasite and results in an epidemic of relapses, the second concerns the number and transmitting capacity of the anopheline vector and results in an epidemic of fresh infections.

The idea of an epidemic of relapses occurring, for reasons unknown, in persons with simple tertian infections is not entirely new. As bearing on this point and also on the inherent periodicity of the parasite, I should like to refer to one of the studies in the treatment of malaria done by workers at Liverpool during the war. It is in the *Annals* of the School in the year 1918, and is entitled

“ A factor hitherto overlooked in the estimation of the curative value of treatments of malaria.”*

Two groups of cases treated identically and microscopically followed for the same period, gave strikingly different relapse rates. In an endeavour to account for this peculiar result investigation was made into several factors which might account for it. The relative numbers in the two groups, the length of time since first infection, the length of time between arrival in England and this treatment, the question of the source of the strain of parasite, whether from Greece or Africa, the strength of the quinine solution and the mode of administration were all carefully scrutinised and shed no light on the matter. The authors were driven finally to the conclusion that the season of the year had a preponderating influence. One group of cases treated and observed in the period July to September gave a relapse rate of only 38 per cent., the other group treated and observed in the period January to April gave a relapse of no less than 94 per cent. The meteorological reports for the periods were examined but the only factor which appeared at all closely correlated with this seasonal difference in relapse rate was temperature ; the lower the mean daily temperature the higher the relapse rate. The percentage of atmospheric humidity did not appear to bear any significant correlation.

Turning now to another point, it is an excellent thing that it is becoming increasingly appreciated that our real medical difficulty in the tropics is the large rural populations. For many years some of us have been directing attention to this fact, and I am glad to see that Colonel GILL gives it prominence. He makes one statement, however, with which I, and I hope the majority of those present, will entirely disagree ; he says of the Ceylon epidemic :—

“ I shall only refer to its disastrous effects in order to emphasise the powerlessness of modern medical science to prevent the outbreak of malaria epidemics or to check materially their courses.”

My reply to this statement is that with few exceptions such as those referred to by Sir MALCOLM WATSON to-night, broadly speaking, modern medical science has never yet had any opportunity of showing what it can effect in the prevention of malaria and other tropical diseases. Let us consider that in the Ceylon epidemic the cost of relief measures alone is put at £350,000. I venture to make the assertion that if modern medical science had had at its disposal any sum of this order of magnitude for the prevention of malaria in Ceylon, the figures of morbidity and mortality in 1934 would have been vastly different from what they unfortunately were.

While, therefore, I heartily congratulate Colonel GILL on his most interesting dissertation, I trust that we shall some day have the pleasure of hearing him repudiate his present pessimistic views on what modern medical science can achieve in the way of prevention, if proper facilities and adequate means are provided.

* *Ann. Trop. Med. & Parasit.*, (1918), xii, 201.

Sir Weldon Dalrymple-Champneys : I was in Ceylon during the worst of the epidemic, and went over the island with Dr. BRIERCLIFFE, the Director of Medical and Sanitary Services there. All the points Colonel GILL has made to-night have come home to me very much. In the joint paper by Dr. BRIERCLIFFE and myself, which no doubt many of you heard at the Royal Society of Medicine, we recounted the chief events in the epidemic, and gave what we considered to be the most important factors in the production of that epidemic. But Colonel GILL has to-night gone, in a more detailed way, into the aetiology of the epidemic, and has produced some very interesting theories. Unfortunately, owing to lack of time, he did not elaborate the latter subsidiary part of his theory concerning sun-spots, but those who have read his paper will have seen it.

I myself—and am sure I can speak for Dr. BRIERCLIFFE also in this matter—would be the last to pretend that this epidemic, or any epidemic of malaria of this kind, can be easily explained or that all the factors are known, and those who have discussed Colonel GILL's paper have made that clear. But, at the same time, I feel that the factors which were suggested by Dr. BRIERCLIFFE in his Report, and by us in our joint paper, as being the principal ones, were in reality the most important factors, though they will not account for all the phenomena which occurred. The three most important and interesting, perhaps, are (1) the encouraging of the breeding of *Anopheles culicifacies* owing to the drought and the consequent pools in the rivers ; (2) the fact that the population of the Wet Zone was very little " salted " to malaria ; and (3)—a point to which Colonel GILL did not refer—the important fact that these people were under-nourished, very many of them grossly so, owing to the partial failure of the paddy crop. I think I am right in saying that Colonel GILL's theory is founded on the assumption that there was an initial epidemic of relapses but the evidence for this seems to me a little thin in places. I have not time now to go into the reasons why I think that ; but there is one point which perhaps he can explain, which seems to be a contradiction in his paper. In one part of the paper he says " The conclusion is inevitable that the children escaped infection almost completely during the first 4 weeks of the epidemic," and from that he concluded that the morbidity amongst adults must have been due, mainly, to relapses. But in a previous part of his paper he said, " At the commencement of the epidemic infants and young children were mainly involved." I am sure there is a simple explanation of that apparent contradiction.

The only other thing I intended to say has been already said, and it is that we should not be satisfied with our present knowledge of malaria, or our present methods of combating it ; I am sure that is what Colonel GILL meant by the remarks which have been described by some as pessimistic. I think they were only intended to stimulate workers on this subject to go forward, and not to rest content with the knowledge we already have. There is no doubt that we must concentrate on research in this matter, and no one is more aware of the importance of that than Dr. BRIERCLIFFE and myself.

Professor P. A. Buxton : Colonel GILL says that the onset of the epidemic was explosive and that one could frequently point to a particular day in the records of a hospital. This is clearly a most important and puzzling observation (whatever may be the interpretation of it). I think that a considerable body of fact should be published showing the daily admissions, either for all diseases or for malaria, in at least a dozen hospitals. At the same time may I point out that the point seems to have escaped the notice of BRIERCLIFFE, whose Charts 4 and 5 and Table 17a in his official report, appear to show a gradual rise, working up to a peak during a period of several weeks. I admit that BRIERCLIFFE's charts may fail to show a lot of important, fine points, because the figures are tabulated by weeks and presented for rather large areas, not for individual hospitals. I do not know whether it would be possible for Colonel GILL's paper to be expanded in this one point.

Colonel Gill (in reply) : The hour is late and I fear that in the time at my disposal it will not be possible to deal adequately with all the points that have been raised by previous speakers. Certain remarks in the introduction to my paper have been adversely criticised by Sir MALCOLM WATSON. I think, however, he has misunderstood the drift of my remarks, but, as Sir WELDON DALRYMPLE-CHAMPNEYS has corrected his misapprehension, I need say little more on the subject.

In my opening remarks, I stated that it was not my intention, in stressing the importance of research, to depreciate what has been achieved in the past or is being attempted in the present to control malaria in the tropics, but that I felt that more knowledge was necessary regarding the epidemiology of malaria, and more particularly the life-cycle of the malaria parasite, in order to broaden the basis of anti-malaria measures and to increase their effectiveness.

As an example of the restricted scope of existing methods I said that it was not possible to put forward at present any practicable scheme for controlling malaria in the very extensive hyper-endemic area in Ceylon. I understood from conversation with Sir MALCOLM that he agreed with me on this point, but he now shakes his head, so apparently he does not. But here are his own words in his speech this evening : " And I would urge that the research I have outlined briefly should include the two-thirds of Ceylon which is now submerged in jungle ; I believe that research will show a way to reclaim this land."

As this is one of the recommendations contained in my official report to the Ceylon Government, I naturally endorse it, but I confess I am at a loss to understand what Sir MALCOLM's position is in this matter. My position is that, although much can be done under favourable conditions, we shall never be able to attack successfully the great problem presented by rural malaria in the tropics until we know more than we do at present regarding the epidemiology of malaria and more especially the life-cycle of the malaria parasite.

But the subject of discussion this evening is the cause of malaria epidemics,

and more especially the circumstances responsible for the sudden outburst of sickness at the commencement of the epidemic. My conclusion that the initial explosion is due to an "epidemic of relapses" is as startling as it was to me wholly unexpected, and it is, of course, necessary that it should be critically examined.

I, therefore, awaited with great interest and some trepidation to hear the views of Sir RICKARD CHRISTOPHERS and Colonel JAMES. I think that the statement of Sir RICKARD that a *prima facie* case calling for serious consideration has been made out goes as far as is justifiable at present. He asked if I could state whether the malaria cases at the commencement of the epidemic were clinically milder than at a later period. I did not specifically investigate this point, but I think that it can confidently be assumed that there were few serious cases during the first few weeks, because at this period there were practically no deaths either amongst adults or children. During the second wave in April 1935, which I witnessed, most of the cases appeared to be mild relapses. Sir RICKARD suggested that mild primary infections might occur at the commencement of the epidemic as the result of infection with a small dose of sporozoites; this would explain their mildness, but it does not account for the sudden and widespread outbreak of sickness at the commencement of the epidemic.

I was much interested in Colonel JAMES's account of the drought epidemic of malaria in Java, but, in reply to another remark, should like to make it clear that I do not regard the great prevalence of *A. culicifacies* in the epidemic area as of no importance; on the contrary, an abundance of good carriers is obviously essential to the occurrence of a wave of new infections; but if the conclusion is correct that the epidemic was initiated by an "epidemic of relapses," then it is clear that, *so far as the initial explosion is concerned*, the presence or absence of anophelines was immaterial.

Colonel JAMES mentioned an interesting suggestion of Dr. DE VERTEUIL, that the explosive outbreak might be due to the mass migration of infected anophelines, but this view fails to explain the almost complete absence of sickness and mortality amongst children during the first 5 weeks of the epidemic, unless it be assumed that the immigrant anophelines selected adults *alone* for attack during the first few weeks of the epidemic.

I should like to thank Professor BLACKLOCK for calling my attention to the paper published in the *Annals* of the Liverpool School of Tropical Medicine entitled "A factor hitherto overlooked in the estimation of the curative value of treatments of malaria," which I remember reading but which I had forgotten. Unless I misunderstood him, Professor BLACKLOCK holds the view that, given reasonably adequate funds, malaria epidemics can be prevented. Everything possible was done by the Medical Department of Ceylon to mitigate the effects of the recent epidemic, but I must plead guilty to being a pessimist, in so far as I believe that, in the present state of scientific knowledge, it is not possible to prevent the occurrence of these epidemics, but I am optimistic enough

to believe that, when the gaps in our knowledge of the epidemiology of malaria have been filled, the prevention of malaria epidemics may become a practicable proposition.

I appreciate the kind remarks of Sir WELDON DALRYMPLE-CHAMPNEYS. He speaks with first-hand knowledge of the epidemic, and with his and Dr. BRIERCLIFFE'S views I am in general agreement, except that, as the result of my study of the Ceylon epidemic, I do not consider that the cause of the epidemic can be attributed primarily to the abundance of anophelines in the epidemic area.

I agree, of course, that famine played a part in increasing mortality during the epidemic (as also did ankylostomiasis), but, so far as the mechanism of the epidemic is concerned, I regard the famine as an incidental consequence of the preceding drought, but otherwise of little or no epidemiological significance.

In regard to an apparent contradiction in my paper, to which Sir WELDON drew attention, I should explain that, in stating that infants and children were mainly involved at the commencement of the epidemic, I was referring to the commencement of *mortality*, and not to the commencement of morbidity, which started some 5 weeks earlier.

Professor P. A. BUXTON has suggested that more data might have been given in my paper in support of the observation that the epidemic broke out so suddenly that its date of onset could often be fixed to a day. I have given daily figures in respect of six hospitals, but I find, on referring to my notes, that in most cases I have only recorded, what was very clear to me at the time, the date of the first sharp rise. The explosive character of the outbreak is, however, clearly apparent in Dr. BRIERCLIFFE'S chart (Fig. 5, p. 439) ; for it is obvious that, when a very sharp rise occurs during a single week (as the chart shows to have occurred in the main epidemic area) the onset must have been extremely abrupt.

My time is up, so I will end by stating that I did not expect that the views I have put forward this evening regarding the mode of onset of malaria epidemics would or could gain immediate acceptance. If this theory be correct it carries with it implications of far-reaching importance, and it may be hoped therefore that further investigations will be made so that the truth may be ascertained within a reasonable time.

MODIFICATION OF THE VIRULENCE OF YELLOW FEVER VIRUS BY CULTIVATION IN TISSUES *IN VITRO*.

BY
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HAAGEN and THEILER (1932) have previously reported successful cultivation experiments with a neurotropic strain of yellow fever virus. They were able to grow the virus in a medium of minced chicken embryo tissue and serum-Tyrode solution contained in Carrel flasks. After more than one hundred passages in this medium, HAAGEN (1933) reported that the virus still retained its essentially neurotropic properties. Similar efforts to cultivate strains of pantropic or natural yellow fever virus did not meet with success (HAAGEN, 1934).

During the past five years a considerable amount of evidence has accumulated to show that a strain of yellow fever virus which has been "fixed" for mouse brain and behaves in a similar way in the nervous tissues of other susceptible animals, represents a greatly modified form of yellow fever virus. While still possessing minor viscerotropic properties it exhibits a behaviour remarkably different from that of the original virus. The natural, or pantropic, virus is capable, in the completely susceptible host (man, monkey, hedgehog), of attacking tissue of ectodermal [brain (PENNA, 1935), (FINDLAY and STERN, 1935)], mesodermal (kidney, myocardium, spleen, lymph gland) or entodermal (liver) origin. The yellow fever virus which is "fixed" for nervous tissue and characterized by major neurotropic properties, if introduced extraneurally into the totally susceptible animal, for example *Macacus rhesus*, is capable of one or both of two types of pathogenesis: (1) a benign infection probably originally confined to the haematopoietic tissues with subsequent invasion of the bloodstream (THEILER, 1935); (2) yellow fever virus encephalitis (DAVIS, LLOYD, FROBISHER, 1932; FROBISHER, 1933; FINDLAY, 1934; THEILER and HUGHES, 1935; THEILER and WHITMAN, 1935a, 1935b). Possibly, too, it might produce classical yellow fever, but the evidence for this is not yet convincing. It is, however, important to note that FINDLAY and CLARKE (1935) have recently

shown that repeated passage of a neurotropic strain through the livers of susceptible monkeys produces a reversion of the virus to the pantropic type.

The limited environmental conditions which the pantropic virus experiences during a continued life in an animal offering principally a susceptible nervous tissue, for example the mouse, leads to a reduction of the viscerotropic and to an enhancement of the original neurotropic properties. As HAAGEN (1933) has shown, this enhanced neurotropic property of the mouse brain-adapted virus is maintained even after 100 passages of the multiplying virus in chicken embryo tissue *in vitro*. However it is conceivable that this neurotropic virus of environmentally reduced potentialities would be capable of lesser alteration when removed to an unfavourable restricted environment than would the original multipotential pantropic virus. The pantropic yellow fever virus submitted to the same or a different environment for growth and reproduction might show variation in behaviour in one or more different ways. Early evidence had already been gained in support of this view when a more critical review of the experiments of RIVERS and WARD (1933) upon the cultivation of vaccinia virus added further weight to the belief in the possible occurrence of a change in a virus by growth under artificial environmental conditions. The latter observers noted that vaccinia virus of calf lymph origin grown for 2 years in a medium of chicken embryo tissue and Tyrode's solution induced little or no reaction in the skin of rabbits although it still gave rise to typical vaccinal pustules in man. They expressed the belief that the change in the activity of the virus for the rabbit was not due entirely—and perhaps not at all—to a gradual diminution in the amount of virus in successive sets of cultures, but to some alteration in the character of the virus itself.

An essentially similar view of the importance of environment as a determinant of the properties of a virus has recently been advanced by FINDLAY and CLARKE (1935) who, on the basis of environmental experiments with yellow fever virus, suggested that its character is largely dependent on the particular substrate on which it is grown. In a further experimental trial of the truth of this concept FINDLAY (1935) has passaged both the neurotropic and viscerotropic (pantropic) yellow fever viruses through a transplantable mouse carcinoma. In this environment of young, actively growing, relatively undifferentiated cells of the mouse, FINDLAY has already gained some evidence that the pantropic virus is losing its viscerotropic activity. As an unfavourable substrate for a pantropic virus the mouse carcinoma tissue is quite analogous to the mouse embryonic tissue medium to be described later.

It is the purpose of this paper to describe the methods of cultivation of the pantropic yellow fever virus and to illustrate experimentally the alterations which have been observed in the properties of this virus during prolonged growth *in vitro*. A brief account is also given of the advantages which may be taken of these changes in perfecting the means of immunization of man against yellow fever.

METHODS AND MATERIALS.

Strains of Yellow Fever Virus under Cultivation.—The following three strains of yellow fever virus have been cultivated from animal sources during the course of this work :

1. Asibi pantropic : This strain was isolated in June, 1927, by BAUER and MAHAFFY from a Negro in the Gold Coast (SAWYER, 1931). It was used for cultivation after 53 monkey passages, with intermittent periods of life in *Aedes aegypti* and preservation in the desiccated state, approximately $6\frac{1}{2}$ years after its isolation. In *Macacus rhesus* this virus produces more than a 95 per cent. mortality from yellow fever 3 to 7 days after inoculation. For information regarding the pathogenesis of this strain reference should be made to the paper by STOKES, BAUER and HUDSON (1928). A complete history of the Asibi virus utilized for cultivation, from the time of its isolation from Asibi to the beginning of cultivation, is summarized in Table II.

2. French neurotropic of 305 mouse brain passages : This is a strain of yellow fever virus originally isolated in December, 1927, by MATHIS, SELLARDS and LAIGRET (1928) from a Syrian in Senegal. In 1929, after having undergone several monkey passages, the virus was adapted to mouse brain by THEILER (1930).

3. French neurotropic of 105 mouse brain passages : This virus is of the same origin as the preceding but of considerably earlier mouse passage. The same strain of an approximately equal number of mouse passages has been used routinely with complementary immune serum for the immunization of man in these laboratories. For information regarding the pathogenesis of the second and third strains of source virus reference should be made to the papers by LLOYD and PENNA (1933), SELLARDS (1931), DAVIS, LLOYD and FROBISHER (1932), FINDLAY (1934), and THEILER and HUGHES (1935).

Method of Cultivation.—The methods of virus cultivation to be described are essentially similar to the technique used by RIVERS (1931) and RIVERS and WARD (1933) for the propagation of vaccinia virus *in vitro*. The culture material was usually contained in Erlenmeyer flasks and consisted of three components : (1) the fluid medium—usually Tyrode's solution containing 10 per cent. by volume of normal human or monkey serum ; (2) the tissue—small pieces of tissue made up of living cells ; (3) the virus component—virus-containing inoculum or supernatant fluid from the corresponding previous subculture.

Tissues.—Mouse embryonic tissue was obtained from pregnant mice of a strain R susceptible to yellow fever virus. The abdomen of the pregnant animal was cleansed with iodine and alcohol ; the skin was reflected and the abdominal wall was washed with alcohol which was allowed to evaporate completely. The peritoneal cavity was incised with dry sterilized scissors and the uterus removed intact to a sterile petri dish. The uterus was opened ; the foeti were taken out and washed four times in 0.9 per cent. sodium chloride solution. The whole embryos were minced together in fine pieces 1 to 2 mm. square with scissors or with a sterile mincing apparatus.

Chicken embryonic tissue was obtained from eggs incubated 9 to 11 days at 37° C. by the method of CARREL and RIVERS (1927). The embryos were washed four times in normal saline solution and minced in fine pieces. Chicken embryo dermis was obtained by reflecting the skin from several embryos, washing in 0.9 per cent. sodium chloride solution and cutting in small squares with scissors. Chicken embryo brain was prepared

by dissecting the brain from the embryo with sharp scissors and forceps. Chicken embryo tissue without the central nervous system was obtained by widely cutting away the brain and spinal column.

The testicular tissues of various animals were obtained in a manner similar to that which had been described for mouse embryos, care always being taken to remove the testes through the peritoneal cavity. The susceptible Swiss strain of mice was used as source of testicular tissues. In order to lessen the chances of contamination from bacteria, testes from one mouse were used for each two flasks; testes from one guineapig for each five flasks. All testicular tissues were minced with scissors.

Fluid Medium.—Tyrode's solution prepared according to the following formula and sterilized by filtration was used: NaCl, 8 gm.; KCl, 0.2 gm.; CaCl₂, 0.2 gm.; MgCl₂, 0.1 gm.; NaH₂PO₄, 0.05 gm.; NaHCO₃, 1.0 gm.; glucose, 1.0 gm.; water to the amount of 1,000 c.c. Except in one series of cultures where the neurotropic virus was grown for long periods of time in a medium consisting only of minced chicken embryo tissue and Tyrode's solution, 10 per cent. of normal serum was always added to the Tyrode's solution immediately before filtration. The addition of the small proportion of serum permitted a longer survival of the extracellular virus. This factor was important since the supernatant fluid after sedimentation of the cells was employed throughout this work as source of virus. Normal serum taken from rhesus monkeys or man was invariably used after having been tested for the absence of protecting properties against yellow fever virus.

Containers.—50 c.c. and 25 c.c. Erlenmeyer flasks (Pyrex), usually the former, were routinely employed. A total of 5.0 c.c. of fluid was added to the larger flasks and 3.0 c.c. to the smaller. We have determined, however, that larger Erlenmeyer flasks of 250 c.c. capacity with correspondingly larger amounts of medium (25 c.c.) can be employed to obtain equally good virus multiplication.

Preparation of Cultures.—Approximately 0.1 gm. of minced tissue was used for each 5 c.c. of serum-Tyrode solution. The tissue suspended in its own fluid, or, if necessary, in a little serum-Tyrode solution, was added to the fluid medium, which was measured into each flask before the addition of the virus component. The virus-containing portion of the original cultures consisted of unfiltered infective monkey serum or filtered infective mouse brain suspension. It equalled 1.0 c.c. in amount in the 50 c.c. Erlenmeyer flasks and 0.5 c.c. in the 25 c.c. flasks. Subcultures were made by transfer of the supernatant fluid after sedimentation of the cells in centrifuge tubes from the old cultures to flasks containing new medium. The flasks were tightly stoppered with corks wrapped in lead foil to confine evaporation to the moist chamber within the flask. All containers were numbered and subcultures were always made from flask to flask of corresponding number. This practice in our experience has very rarely led to dying out of virus while it has insured greatly against the widespread bacterial contamination which is prone to occur when a plan of subinoculation from pooled cultures is followed. All cultures were incubated at 37° C. for 3 to 4 days before transferal.

Tests for Sterility of the Cultures.—Careful inspection of the normally clear supernatant fluid was usually sufficient to detect any gross bacterial or mould contamination. In doubtful cases smear preparations were stained for bacteria; and from all cultures from time to time 0.1 c.c. of the supernatant fluid of each flask was inoculated in a tube of pneumococcus broth, which was incubated and subsequently searched for bacterial growth.

Tests for the Presence of Virus.—At the time of each subculture the supernatant fluid from groups of five flasks was tested for infectivity by the intracerebral inoculation of groups of six mice. Both pantropic and neurotropic strains of yellow fever virus regularly produce encephalitis in mice when inoculated into the brain. Occasionally each individual culture of a series was tested for the presence of virus. At a time when the results could be read on such infectivity tests occasional flasks which did not show the presence of virus were discarded and replaced from actively growing cultures. Frequently the content of virus in the pooled supernatant fluid from a series of cultures was approximately determined by the intracerebral inoculation of groups of mice with serial decimal dilutions of the culture fluid. The titre of virus in the supernatant fluid as determined by this method varied in different series and at different times from 10³ to 10⁶, usually being nearer the upper end of this scale.

Multiplication of the Virus in vitro.—Inasmuch as the virus was diluted five-fold in the first culture and underwent a five-fold dilution in each subsequent subculture, and since the blood serum or mouse brain suspension used as source of virus was not infectious in dilutions greater than 10-9, it soon became obvious that yellow fever virus was multiplying in each series of cultures.

Further evidence of this kind was obtained early in the course of the cultivation experiments by a daily titration of the amount of virus growing in flasks containing mouse embryonic tissue and serum-Tyrode solution at the time of the 22nd subculture in this medium. From each of a series of ten cultures, at 24 hour intervals for 8 days, 0.25 c.c. of the culture fluid was removed and pooled. The withdrawn fluid was replaced with 0.25 c.c. of serum-Tyrode solution. The culture fluid pool at each daily interval was centrifuged and the supernatant fluid was made up in serial decimal dilutions from 1/10 to 1/1,000,000 in 10 per cent. normal monkey serum-Tyrode solution. In preparing each dilution 1.0 c.c. of the lower dilution was uniformly mixed with 9.0 c.c. of the diluent. A fresh pipette was always used for each successive dilution. Each dilution was inoculated in 0.03 c.c. amounts intracerebrally into each of a group of six mice, which were observed for a period of 21 days. The titre of the virus in every specimen of supernatant culture fluid pool was indicated by the death of mice of typical yellow fever virus encephalitis in the higher dilutions. Readings of the titre for each 24 hour period in this experiment, as well as all later titres of either virus or antibody reported in this paper, were determined according to the method of MUENCH (1934)*, and are recorded always as whole numbers which represent the number of times to which the virus or antibody was required to be diluted to give an end point. In Graph 1 is illustrated the curve of virus concentration on successive days of cultivation in mouse embryonic tissue and serum-Tyrode solution. It can be readily learned from a study of the Graph that the concentration of virus after 24 hours was 165, after 48 hours 3,000, after 72 hours 40,000, after 96 hours 40,000, after 120 hours 4,500, after 144 hours 2,500, after 168 hours 3,000, and after 192 hours 1,400.

Preservation of the Virus.—At frequent intervals, usually every five passages, during the cultivation of each strain, the virus was frozen and desiccated in vacuo in the solid state according to a method previously described for the preservation of yellow fever virus over long periods (SAWYER, LLOYD and KITCHEN, 1929). The supernatant fluid from individual cultures or pools of cultures was diluted with an equal quantity of normal serum, human or monkey, and frozen and desiccated in 1.0 c.c. amounts in Wassermann tubes. The tubes were removed from the desiccators when their contents were completely dry, sealed and stored at 4° C. Culture virus preserved and stored by this technique can be readily rehydrated by the addition of the amount of distilled water lost in evaporation and used as the source of new cultures. The method provides in the first instance a practical means of insurance against loss of strain by bacterial contamination, and in the second, the opportunity of reinvestigating or of utilizing the virus at any stage of the cultivation experiment.

CULTIVATION OF PANTROPIC YELLOW FEVER VIRUS.

CULTIVATION IN EMBRYONIC TISSUES.

Cultivation in Whole Mouse Embryonic Tissue.—Mouse embryonic tissue is the only medium in which we have succeeded in securing an original "take" of the pantropic strain of virus of animal origin. After a varying number of

*The method of MUENCH depends upon using as an end point in a titration either of yellow fever virus or of protecting serum antibodies the dilution at which half the mice survive and half die. From the mortality ratios or protection ratios of the groups of mice (according to whether virus or antibody respectively is being titrated) inoculated with the several dilutions, the theoretical dilution which would effect a 50 per cent. mortality is mathematically calculated. This calculated dilution is taken as the titre of the virus or antibody.

passages in mouse embryonic tissue, this virus has been transferred to, and grown with comparative ease in, other tissues such as chicken embryo and mouse testis. However, it has not been possible, in a limited number of attempts, to transmit the virus directly from the animal host to chicken embryo or mouse testicular tissue. It is probable that further efforts, attended by as yet unknown optimal conditions, might have been productive of original growth in such media. Although an initial attempt to cultivate the virus in mouse embryonic tissue and serum-Tyrode solution in Erlenmeyer flasks resulted in loss of the virus in the second subculture, a second experiment utilizing the same technique readily effected propagation of the strain which has now been grown for 50 passages in quadruplicate series and for more than 130 passages in duplicate series.

During the prolonged period of cultivation *in vitro* the mouse embryonic tissue strains have shown a progressive diminution in their virulence for the entodermal and mesodermal tissues of *Macacus rhesus*, but an unaltered or slightly diminished virulence for nervous tissues. On the other hand, when inoculated intracerebrally in mice the virus constantly produced encephalitis with the characteristically prolonged incubation period of the original pantropic virus of monkey origin.

Cultivation in Whole Chicken Embryonic Tissue.—In one series of cultures of the pantropic virus in mouse embryonic tissue and serum-Tyrode solution, whole chicken embryonic tissue was substituted for mouse embryo in the nineteenth subculture. This strain has now been carried in chicken embryo tissue for more than 130 additional subcultures (more than 150 subcultures in flask cultivation).

Like the strain of pantropic yellow fever virus grown in mouse embryonic tissue the strain cultivated in chicken embryo has lost greatly in entodermotropic and mesodermotropic virulence with prolonged cultivation. The original neurotropic properties of the pantropic strain have shown no apparent alteration so far as can be determined by animal infectivity tests.

Cultivation in Chicken Embryo Dermis.—After 28 subcultures in whole chicken embryo tissue and serum-Tyrode solution, chicken embryo dermis was substituted for whole chicken embryo as the cellular medium. This pantropic strain has now been cultivated for 31 passages in this restricted medium. Its pathogenesis in mice has not been noticeably different from that of the strain grown in whole chicken embryo. Its behaviour in monkeys has not been investigated.

Cultivation in Chicken Embryo with Greater or Lesser Proportions of Nervous Tissue.—A strain of pantropic yellow fever virus which had been grown for 56 subcultures in whole chicken embryonic tissue and serum-Tyrode solution was cultivated in parallel series for 27 passages in identical fluid media (serum-Tyrode solution) but in contrasting tissue cells—chick embryo brain and chick embryo tissue from which brain and spinal cord had been dissected away.

Rather surprisingly, the viruses of the parallel series, irrespective of the large or minimal amount of nervous tissue provided for growth and multiplication, evinced an essentially, although not completely, similar behaviour in their pathogenesis for mice and monkeys. At the end of the period of cultivation both strains of this virus produced fatal encephalitis when inoculated intraspinally in *M. rhesus*. The supernatant fluid from equal numbers of flasks (10) of each subculture of both strains of virus was inoculated intracerebrally in parallel groups of mice (usually 6, occasionally 60). The average period from inoculation of the virus to the death of the mice was computed for each strain for 27 subcultures. This interval varied from 6·7 to 9·0 days for the virus grown in brain tissue, and from 7·4 to 10·5 days for the virus grown in embryo tissue without brain and cord. The average periods for the whole series were, however, closely similar, being 8·1 days for the former and 8·4 days for the latter.

CULTIVATION IN ADULT TISSUES.

Cultivation in Mouse Testicular Tissue.—A strain of pantropic yellow fever virus, grown for 27 subcultures in mouse embryonic tissue, was used as source of virus for a series of cultures in adult mouse testicular tissue and serum-Tyrode solution. The virus grows well in the latter medium and it has now been carried through more than 110 passages.

The strain of virus cultivated in mouse testicular tissue originating from an altered strain of pantropic virus has further lost in its ability to provoke fatal or even clinically recognizable yellow fever in the monkey. Its original neurotropic properties as evidenced by its inoculation into the nervous tissues of mice or monkeys have remained apparently unchanged. This strain of virus after 37 subcultures in mouse testicular tissue *in vitro* was inoculated in the testes of living mice, through which it has been transferred at weekly intervals for more than 20 passages. At the time of the 10th passage virus was demonstrable in the testicular tissue in a titre of 220,000. Since a limited number of previous and contemporary efforts by ourselves and others to pass the original Asibi pantropic strain of the virus through the testes of living mice did not meet with success, this demonstrated multiplication *in vivo* may or may not be indicative of a greater or lesser amount of specific tissue adaption of the virus by the technique employed.

Cultivation in Guinea-pig Testicular Tissue.—An originally pantropic strain of the virus, after cultivation for 27 passages in mouse embryonic tissue and 41 passages in mouse testicular tissue, was transferred in the same serum-Tyrode medium to guinea-pig testicular tissue where it grew readily. This virus was lost in the 20th subculture through bacterial contamination from the guinea-pig tissue. The strain was re-established from the 70th subculture of the *in vitro* strain in mouse testis and has for a second time been passaged through 55 subcultures in guinea-pig testicular tissue.

The characteristics of this strain as yet determined do not show appreciable differences from the pantropic virus grown in mouse testicular tissue. Both show the characteristically long incubation period for mice, when inoculated intracerebrally, of the parent mouse embryonic strain; both produce encephalitis in monkeys when introduced into the spinal canal, but fail to produce yellow fever when injected by extraneural routes.

Attempts of Cultivation in Testicular Tissues of Resistant Animals.—One attempt to transfer the pantropic strain cultivated for 35 subcultures in mouse testicular tissue to a similar medium in which albino rat testis was substituted for mouse testis resulted in loss of the virus after the second passage.

In a similar effort to subinoculate the mouse testicular strain of 53 passages into rabbit testicular tissue suspended in the same fluid medium the virus did not survive beyond the first culture. Both the rabbit and the rat are animals resistant to inoculation of pantropic or neurotropic strains of yellow fever virus.

CULTIVATION OF NEUROTROPIC YELLOW FEVER VIRUS.

As controls on the cultivation experiments with pantropic strains of yellow fever virus two neurotropic strains, one of relatively early and one of relatively late mouse passage were grown *in vitro* by techniques identical with or similar to those used for the cultivation of the pantropic viruses. Utilizing the methods described, and employing Erlenmeyer flasks, each of 3 attempts to grow neurotropic strains of the virus in either chick or mouse embryonic tissue and serum-Tyrode solution met with success.

Cultivation in Whole Mouse Embryonic Tissue.—The French strain of yellow fever virus after 305 passages in the brains of mice was grown for 55 subcultures in a medium consisting of minced whole mouse embryo and serum-Tyrode solution. The strain as judged by its pathogenesis in mice behaved from the beginning to the end of the cultivation period as fixed neurotropic yellow fever virus. Nevertheless, it should be noted that the period from inoculation to death in mice following the intracerebral injection of the cultivated neurotropic virus was on the average about 2 days longer than that of the original mouse passage virus. This was true during the beginning as well as at the end of the period of cultivation.

Cultivation in Whole Chicken Embryonic Tissue.—Two neurotropic viruses have been cultivated. The French strain of yellow fever virus after 305 passages in mice was grown for 16 subcultures in a medium of minced chicken embryo tissue and serum-Tyrode solution. At this stage of the experiment the virus was lost through the occurrence of a uniform bacterial contamination. This cultivated strain was re-established in chicken embryo tissue at the 21st subculture from virus of the same origin grown for 20 subcultures in mouse embryonic tissue. It has now been cultivated for more than 140 passages in the same medium. The strain has behaved consistently from the beginning

to the end of the cultivation series as a fixed neurotropic virus. At each subculture it was injected into the brains of mice, in which the period from inoculation to death was on the average 2.5 days longer than that of the corresponding virus of mouse passage.

After 46 transfers in a medium of chicken embryo tissue and serum-Tyrode solution this neurotropic strain of the virus has been cultivated for more than 86 passages in a medium in which Tyrode's solution was substituted for the serum-Tyrode solution. This virus has evidenced an essentially similar behaviour to the corresponding strain in chicken embryo tissue and serum-Tyrode solution.

A second strain of the French neurotropic virus, cultivated after 108 passages in mouse brain has been grown for more than 120 subcultures in a medium of chicken embryo tissue and serum-Tyrode solution. Although this cultivated strain of virus, when inoculated intracerebrally at each subculture into mice, has behaved as a relatively fixed neurotropic virus, the average period from inoculation to death throughout the series has been 1 to 1.5 days longer than those of the more fixed (305 mouse passages) neurotropic strains cultivated in the same medium. Moreover, this average period is 3 days longer than that of the original mouse passage strain (108 mouse passages).

Cultivation in Chicken Embryo with Greater or Lesser Proportions of Nervous Tissue.—The strain of neurotropic yellow fever virus which had been cultivated after 108 mouse passages and grown for 46 subcultures in whole chicken embryo and serum-Tyrode solution was propagated in parallel series for 27 passages in the same fluid medium but in different tissue cells—chicken embryo brain and chicken embryo from which the brain and spinal cord had been dissected away. The viruses of the parallel series, despite the great differences in the amount of nervous tissue provided for growth, showed no great variation in their pathogenesis for mice when inoculated intracerebrally. The average period from inoculation to death was 7.5 days for the virus cultivated in chicken embryo without brain and cord tissues and 7.1 days for the virus grown in chicken embryo brain.

PATHOGENESIS IN ANIMALS OF CULTIVATED STRAINS OF YELLOW FEVER VIRUS.

PATHOGENESIS OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN MOUSE EMBRYONIC TISSUE.

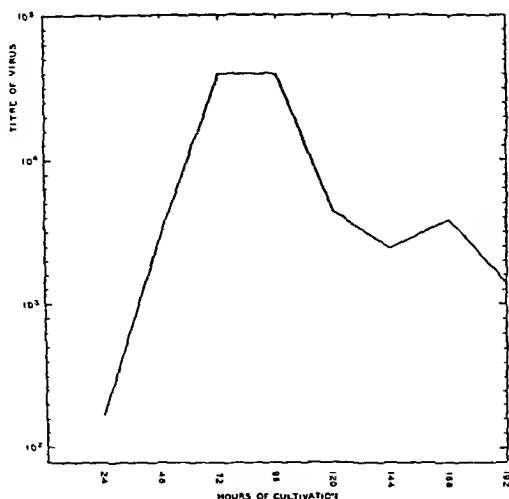
Since all strains of cultivated virus were inoculated intracerebrally in mice at the time of each subculture, a comparative study of the period from inoculation to death during all stages of each cultivation experiment gives a rather reliable index of the degree of neurotropism of each virus.

In Table I are summarized the average inoculation—death periods of the five principal cultivated strains for each 10 passages during 100 or more subcultures in the selected medium. Averages are not recorded more frequently

than the 10 passage intervals for the reason that marked variations occur from subculture to subculture dependent for the most part upon differences in quantity of virus.

The pantropic strain of yellow fever virus at the time of the commencement of its cultivation in mouse embryonic tissue showed an average inoculation-death period in mice of ± 10.0 days. As may be observed from consulting Table I, the corresponding periods for the first three 10 passage averages were 10.8, 10.9 and 10.5 days. Commencing with the 28th subculture the period of incubation of all cultures was changed from 7 to 3 or 4 days. A study of Graph 1 will reveal the fact that such a change in cultivation technique would

GRAPH 1.—MULTIPLICATION OF YELLOW FEVER VIRUS IN FLASK CULTIVATION.



usually result in the inoculation of about 13 times as much virus in each infectivity test. In close correspondence with this change, the inoculation—death period after the 27th subculture was consistently shorter, the 10 passage averages varying between 8.6 and 9.7 days. The important fact which may be learned from these data is that there was neither significant increase nor decrease in the inoculation—death period during the time of cultivation. Considering this criterion alone the property of neurotropism of the original pantropic virus appears unaltered.

Although infectivity tests in mice yield information which is most valuable because it can be based upon the results of behaviour of the virus in very large numbers of animals, it was believed that for the purposes of this study the observations upon the pathogenesis of the virus in *Macacus rhesus* offered opportunity for a broader appreciation of its biological properties and a closer analogy to the conditions which might obtain in *man*. Admitting that man is an animal

TABLE I.

AVERAGE DURATIONS OF PERIOD FROM INOCULATION TO DEATH IN MICE OF VARIOUS CULTIVATED STRAINS OF YELLOW FEVER VIRUS.*

Number of sub-cultures.	Strain of Virus.					
	Pantropic.			Neurotropic.		
	Mouse embryo.	Chicken embryo.	Mouse testis.	Earlier mouse† passage virus in chick embryo.	Later mouse‡ passage virus in chick embryo.	Later mouse‡ passage virus in mouse embryo.
0-10	10.8 (10.0)§			7.5	6.3 (3.7)§	5.9 (3.7)§
11-20	10.9			7.8	6.3	5.9
21-30	10.5	9.9		7.3	6.1	5.7
31-40	8.8	8.5	9.0	7.8	6.6	6.1
41-50	8.6	9.0	8.7	7.3	6.4	5.9
51-60	8.6	7.9	8.6	7.5	6.0	
61-70	9.2	9.4	8.2	7.7	6.5	
71-80	9.7	8.7	8.6	7.8	6.6	
81-90	8.9	8.6	8.8	7.9	6.6	
91-100	8.9	8.5	8.9	8.6	6.8	
101-110	9.1	8.7	9.4		6.9	
111-120	9.0	8.6	9.1		7.0	
121-130		8.4	9.6		6.3	
131-140			9.4			

Explanation : * Time in days.

† 108 mouse passages.

‡ 305 mouse passages.

§ Average period from inoculation to death for source virus used for cultivation.

less susceptible to yellow fever virus than is the experimental animal *M. rhesus*, interpretation of results obtained in the monkey when applied to man should provide criteria conservatively safe for further investigation of the behaviour of a modified form of the virus used in human prophylaxis.

In Table III are summarized the results of the extraneural inoculation of *M. rhesus* at various stages of the cultivation experiment with the originally pantropic virus cultivated in mouse embryonic tissue and normal monkey serum-Tyrode solution. All animals of the series recorded in Table III were proven susceptible to yellow fever virus, before inoculation, by the demonstration of the absence of protecting antibodies in their blood.

Before studying Table III it is important to remember that the pantropic virus of Asibi strain used as source of virus for the cultivated strain under consideration is one which during monkey to monkey passage kills more than 95 per cent. of animals.

The essential data relative to the behaviour of the Asibi strain of pantropic virus in *M. rhesus* during the 6½ years from its isolation from Asibi to its cultivation in mouse embryonic tissue are summarized in Table II. Although frequently interrupted in its animal passage, the virus during this period was transferred with intermittent periods of life in mosquitoes and preservation in the dried state through 53 passages in rhesus monkeys. Irrespective of whether the transmission was effected biologically by mosquitoes or artificially by syringe inoculation of blood or serum, all of 59 rhesus monkeys so inoculated died of yellow fever. Five animals in the series which would in all probability have died were prematurely killed. Once an American monkey, *Cebus fatuellus*, relatively resistant to the virus, was used for passage. The total calculated period of life in *M. rhesus* before cultivation was 167 days.

A critical review of the results recorded in Table III will reveal the fact that the pathogenicity of the virus during the cultivation experiment may be described separately during four periods.

Arbitrarily the first period may be limited to the behaviour of the virus for the first 10 subcultures, during which time it killed two monkeys of yellow fever when the virus of the 5th and 10th subcultures was inoculated. It is, however, worthy of consideration that while the animal receiving the fluid from the 5th subculture died as promptly of yellow fever as if it had been inoculated with the monkey passage virus, the animal injected with the 10th subculture survived 3 days longer.

In the second period is included the pathogenesis of the virus from the 15th to 25th subcultures, during which time it killed 5 of 10 monkeys of yellow fever, but equally permitted 5 animals to escape death and attain demonstrable immunity.

In the third period is considered the behaviour of the virus between the 25th and 45th passages in cultivation. It is significant that during this time only 1 of 9 monkeys inoculated with the passage virus succumbed to yellow fever, while 8 survived and attained immunity.

In the fourth period are included all animals inoculated with the virus of various passages in mouse embryonic tissue from the 49th to the 109th subcultures. Although in all of these virus was recovered from the blood stream on 2 or more days during the first week following inoculation, 3 of the 17 monkeys inoculated during this period suffered no febrile reaction. The remaining 14 showed fever but in most instances of short duration. Both the duration of fever and the incidence of febrile attacks were less towards the end of the series. None of the 17 presented clinical signs of illness. All animals were subsequently demonstrated to be immune to yellow fever. As evidence against an increased degree of neurotropism in the cultivated virus is the fact that no animal in this series developed yellow fever virus encephalitis.

The results of the inoculation of *M. rhesus* by neural routes with the virus cultivated in mouse embryonic tissue and normal monkey serum-Tyrodé

solution are shown in Table IV. The volume of culture fluid used as an inoculum was invariably 0.5 c.c., although the quantity of virus varied as is shown in the table. All monkeys used in this series exhibited no protecting antibodies for yellow fever virus in their sera at the time of inoculation. As may be seen from an examination of Table IV, all of three monkeys, inoculated intracerebrally with the virus grown in mouse embryonic tissue of the 35th and 82nd subcultures, died with the lesions of yellow fever virus encephalitis. It is, however, worthy of note that two of these animals showed histologically the specific liver lesions of yellow fever. Because of the absence of cerebral injury intraspinal inoculation offers a less crucial test of the neurotropism of a strain of yellow fever virus than does intracerebral inoculation. Of 10 monkeys injected in the spinal canal with the virus-containing culture fluid of various passages, 2 out of 3 inoculated with the virus of the 45th subculture died of yellow fever; one injected with the virus of the 76th passage died of yellow fever virus encephalitis, while 7 animals experienced febrile reactions varying from 0.5 to 4.5 days in duration, but recovered. All intraspinal inoculations were made certain by the previous withdrawal of clear spinal fluid before the injection of virus. Three of the monkeys inoculated with the virus of the 76th subculture were clinically very ill, but recovered. The remaining 4 animals of the 10 inoculated intraspinally were without signs of evident illness. In 6 monkeys of this series the course of circulating virus in the blood was followed by bleeding the animals on alternate days for 8 days and inoculating the serum intracerebrally in mice. In all these monkeys virus was recoverable from the blood from the 2nd to 5th days following inoculation. It was usually present in smaller quantities on the 6th and 7th days. In Table IV, the amount of virus demonstrated in the serum taken on a given day is approximately indicated by the mortality ratio in a group of usually 6 mice injected with the undiluted serum. All monkeys which survived intraspinal inoculation were tested for immunity either by the titration of protecting antibodies in mice or by subsequent inoculation of virulent pantropic virus or by both methods. In 5 recovered animals in which the serum antibodies were titrated one month following inoculation, the titre was 256 or greater (Muench method). The results recorded following the intraspinal inoculation of *M. rhesus* with the pantropic virus cultivated in mouse embryonic tissue stand in striking contrast with those reported by LLOYD and PENNA (1933), following the inoculation of monkeys with "fixed" neurotropic virus by the same route. These workers observed a uniform fatality in all of 23 monkeys receiving the neurotropic virus intraspinally. For purposes of comparison the essential data of these results are summarized in Table V.

While the original strain of pantropic yellow fever virus cultivated in a medium of mouse embryonic tissue and normal monkey serum-Tyrode solution which has been described above has evinced consistently a degree of neurotropism not greater than that of the original pantropic virus of animal

TABLE II.

PATHOGENESIS OF PANOTROPIC YELLOW FEVER VIRUS FOR *M. rhesus* FROM THE TIME OF ITS ORIGIN FROM ASIBI TO THE BEGINNING OF CULTIVATION.

Monkey Number.	Passage in <i>M. rhesus</i> .	Inoculum of Virus.			Febrile reaction.		Final result.	
		Source.	Amount in c.c.	Route.	Days to fever.	Total days of fever.	Death.*	Survival.
L1	1	blood Asibi	2.0	I.P.	4	1	5Y.F.	no
L2	2	blood L1	2.0	I.P.	3	3	6Y.F.	no
L3	3	blood L2		M.T.			8Y.F.	no
L4	4	blood L3		M.T.	3	0.5	4Y.F.	no
L5	5	blood L4		M.T.	3	0.5	5Y.F.	no
L6	6	blood L5		M.T.	4	0.5	5Y.F.	no
L7	7	blood L6		M.T.	3	1.5	8Y.F.	no
L8	8	blood L7	3.0 mosquito suspension	S.C.	4	2	9Y.F.	no
L9	9	blood L8	1.0	S.C.	4	3	8Y.F.	no
L10	10	blood L9		M.T.	4	1	5 killed	
L11	11	blood L10	1.0	S.C.	3	2	5Y.F.	no
L12	12	blood L11	1.0	S.C.	3	1.5	6Y.F.	no
L13	13	blood L12	1.0	S.C.	2	1	4Y.F.	no

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L14	14	blood L13	1.0	S.C.	2	1	3Y.F.	no
L15	15	blood L14	1.0	S.C.	2	1.5	4Y.F.	no
L16	16	blood L15		M.T.	3	1.5	6Y.F.	no
L17	17	blood L16	0.5	S.C.	2	2	7Y.F.	no
L18	18	blood L17		M.T.	3	0.5	6Y.F.	no
L19	19	blood L18	1.0	S.C.	3	0.5	4Y.F.	no
L20	20	blood L19†	1.0	S.C.	3	1.5	4 killed	
L21	21	blood L20†	1.5	S.C.	4	2	7Y.F.	no
L22	22	blood L21	1.0	S.C.	2	1	2 killed	
L23	23	blood L22	1.0	S.C.	3	1	4Y.F.	no
L24	23	blood L22	0.5	S.C.	3	1	4Y.F.	no
S1	24	blood L23, L24†	1.0	I.P.	17	2	20Y.F.	no
S2	25	blood S1†	0.5	I.P.	3	4	7Y.F.	no
S3	26	blood S2	0.0056	S.C.	3	1	4Y.F.	no
S4	27	blood S3	0.5	I.P.	2	0.5	3Y.F.	no
S5	28	blood S4†	1.0	I.P.	2	0.5	3Y.F.	no
S6 (<i>Cebus fauvelus</i>)†	—	blood S5	26.0	I.P.	2	1.5		survival†

Monkey Number.	Passage in <i>M. rhesus</i> .	Inoculum of Virus.			Febrile reaction.		Final result.	
		Source.	Amount in c.c.	Route.	Days to fever.	Total days of fever.	Death.*	Survival.
S8	30	serum S7	1.0	S.C.	2	1	4Y.F.	no
S9	31	blood S8†	0.5	I.P.	2	0.5	3Y.F.	no
S10	32	blood of S9	0.8	S.C.	2	1	4Y.F.	no
S11	33	blood S10†	0.5	I.P.	2	2	7Y.F.	no
S12	34	blood S11	0.25	S.C.	3	0.5	5Y.F.	no
S13	35	blood S12	0.5	I.P.	3	0.5	4Y.F.	no
S14	36	serum S13†	0.5	I.P.	2	2.5	5Y.F.	no
S15	37	blood S14	0.25	S.C.	3	0.5	5Y.F.	no
S16	38	blood S15	0.5	I.P.	2	1	3Y.F.	no
S17	39	blood S16†	0.5	S.C.	2	1.5	4Y.F.	no
S18	40	blood S16,† S17	0.4	S.C.	2	1	5Y.F.	no
S19	40	blood S16,† S17	0.4	S.C.	3	1.5	5Y.F.	no
S20	40	blood S16,† S17	0.4	S.C.	2	0.5	3Y.F.	no
S21	41	serum S18, S19, S20†	0.5	S.C.	4	0.5	7Y.F.	no
S22	41	serum S18, S19, S20†	0.5	S.C.	3	1	7Y.F.	no
S23	42	serum S21, S22†	0.5	S.C.	2	1	4Y.F.	no
S24	42	serum S21, S22†	0.5	S.C.	3	1	4Y.F.	no
S25	42	serum S21, S22†	0.5	S.C.	3	1	5Y.F.	no
S26	42	serum S21, S22†	0.5	S.C.	2	1	5Y.F.	no

S27	43	serum S22, S23†	0-4	S.C.	3	1	5Y.F.	no
S28	43	serum S22, S23†	0-4	S.C.	3	0-5	5Y.F.	no
S29	43	serum S24, S25, S26†	0-5	S.C.	2	1	3Y.F.	no
S30	44	serum S27, S28, S29†	0-4	S.C.	2	0-5	3Y.F.	no
S31	45	serum S28, S29, S30†	0-4	S.C.	3	0-5	5Y.F.	no
S32	46	serum S29, S30, S31†	0-4	S.C.	3	0-5	5Y.F.	no
S33	47	serum S32†	1-0	I.P.	2	1	5Y.F.	no
S34	48	blood and serum S31, S32, S33†	1-5	I.P.	2	1	4Y.F.	no
S35	49	serum S34†	0-5	I.P.	3	0-5	5Y.F.	no
S36	50	serum S35†	0-6	S.C.	2	0-5	3Y.F.	no
S37	50	serum S35†	0-6	S.C.	2	0-5	6Y.F.	no
S38	51	serum S36, S37†	0-5	I.P.	2	1	5Y.F.	no
S39	51	serum S36, S37†	0-5	I.P.	2	-	2 killed	
S40	52	serum S38, S39†	0-5	S.C.	3	1	5Y.F.	no
S41§	53	serum S40	0-5	S.C.	2	-	2 killed	

Explanation : * Days to death recorded before cause of death.

† Desiccated.

Y.F. Yellow fever.

‡ American monkey *Cebus fatuellus*; yellow fever is not fatal to this species.

§ Source of virus for cultivation experiments.

S.C. Subcutaneous.

I.P. Intraperitoneal.

M.T. Mosquito transmission.

TABLE III.
PATHOGENESIS OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN MOUSE EMBRYONIC TISSUE FOR *M. rhesus* WHEN INOCULATED BY EXTRANEURAL ROUTES.

Monkey Number.	Inoculum of virus.			Virus† isolated from blood 1-7 days after inoculation.	Febrile reaction.		Death.*	Final result.	
	Fluid of subculture.	Amount in c.c.	Route.		Days to fever.	Total days of fever.		Presence of serum antibodies.	Response to test inoculation.
W1	5	2.0	I.P.		3	1	4Y.F.		
W2	10	2.0	I.P.		4	2	7Y.F.		
W3	15	2.0	I.P.		5, 9§	3		+	nil
W4	17	2.0	I.P.		3	4.5		+	nil
W5	18	2.0	I.P.		5, 10§	2.5		+	nil
W6	19	2.0	I.P.		4	1.5	7Y.F.		
W7	19	2.0	I.T.		7	2			nil
W8	20	2.0	I.P.		4	1	8Y.F.		
W9	21	2.0	I.P.		3	1	5Y.F.		
W10	22	2.0	I.P.		4	1	6Y.F.		
W11	24	2.0	I.P.		3	5			nil
W12	25	2.0	I.P.		5	1	6Y.F.		
W13	26	2.0	I.P.		5	2			nil
W14	26	2.0	I.P.		6	2			nil
W15	29	2.0	I.P.		6	1			nil
W16	31	2.0	I.P.		2	3			
W17	35	2.0	I.P.		5	3			nil
W18	41	2.0	I.P.			0			nil

W19	45	2.0	I.P.			4	1	7Y.F.		
W20	45	2.0	I.P.				0			nil
W21	45	2.0	I.P.			4	1			nil
W22	49	1.0	I.P.	+		5	3		+	nil
W23	58	1.0	I.P.	+		4	5		+	nil
W24	65	1.0	I.P.	+		6	1		+	nil
W25†	69	2.0	I.P.	+		6	1.5		+	nil
W26†	69	2.0	I.P.	+		6	0.5		+	nil
W27†	76	2.0	I.P.	+		6	3		+	nil
W28	82 & 83	1.0	S.C.	+		3, 7§	1		+	
W29	84	1.0	S.C.	+		9	1		+	nil
W30	84	1.0	S.C.	+		6	2		+	nil
W31	92	2.0	I.P.	+		6	0.5		+	nil
W32	109	1.0	S.C.	+			0		+	nil
W33	109	1.0	S.C.	+		6	1		+	nil
W34	109	1.0	S.C.	+		6, 10, § 12	2		+	nil
W35	109	1.0	S.C.	+			0		+	nil
W36	109	1.0	S.C.	+			0		+	nil
W37	109	1.0	S.C.	+		7	1		+	nil
W38	109	1.0	S.C.	+		9	1		+	nil

Explanation: * Days to death recorded before cause of death.

† The blood of the latter monkeys in this series was tested in mice for the presence of virus at daily or two daily intervals. In the blood of each animal virus was consistently demonstrated on 2 or more days during the first week following inoculation.

nil No reaction.

† Virus present—absence of symbol means no test.

‡ These animals received at the same time 0.5 c.c. of the virus-containing inoculum intraspinally.

§ Separate febrile reactions.

S.C. Subcutaneous.

I.P. Intraperitoneal.

I.T. Intratesticular.

Y.F. Yellow fever.

TABLE IV.

PATHOGENESIS OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN MOUSE EMBRYONIC TISSUE FOR *M. rhesus* WHEN INOCULATED BY NEURAL ROUTES

Monkey number.	Inoculum of virus.		Circulation of virus.										Febrile reaction.		Final result.			
			Serum inoculated in groups of mice. Mortality ratios in mice on following days :															
			Fluid of sub-culture.	Quantity of virus.	Route.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			Day 8	Days to fever.	Total days of fever.	Days to death.
W39	35		I.C.										5	5	13	Y.F.V. encephalitis — Y.F.		
W40	41		I.S.										4	4				nil
W41	45		I.S.										4	1	6	Y.F.*		
W42	45		I.S.										4	2				nil
W43	45		I.S.										2	4.5	8	Y.F.		
W25†	69	17,000MLD.	I.S.	6/6			6/6		2/5		0/3	6	1.5				T256	nil
W26†	69	17,000MLD.	I.S.	6/6			5/5		0/3		0/2	6	0.5				T256	nil

W27†	76	1,700MLD.	I.S.	0/6	6/6	2/4	0/6	6	3			T256	nil
W44†	76	1,700MLD.	I.S.	0/6	5/5	5/6	3/5	5	4	12	Y.F.V. encephalitis		
W45	76	1,700MLD.	I.S.	0/6	4/5	6/6	0/4	5	4.5			T+256	nil
W46	76	1,700MLD.	I.S.	0/6	5/5	6/6	2/6	6	3			T+256	nil
W47	82	8,500MLD.	I.C.					4	2	9	Y.F.V. encephalitis — Y.F.		
W48	82	8,500MLD.	I.C.					4	2.5	10	Y.F.V. encephalitis		

Explanation:

— Presence of circulating virus.

MLD. Minimal lethal dose for a mouse.

Y.F.V. Yellow fever virus.

Y.F. Yellow fever.

* Virus isolated from brain at death.

I.C. Intracerebral.

I.S. Intraspinal.

T. Titre.

nil No reaction.

† These animals received at the same time 2.0 c.c. virus intraperitoneally.

TABLE V.

PATHOGENESIS OF NEUROTROPIC YELLOW FEVER VIRUS FOR *M. rhesus* WHEN INOCULATED BY NEURAL ROUTES.

Monkey number.	Inoculum of virus intraspinally.		Febrile reaction.		Final result.		
	Source.	Amount in e.e.	Days to fever.	Total days of fever.	Death.		Recovery.
					Days to death.	Cause of death.	
B1	B-N filtrate mouse brain†	0.5	5	3	8	Y.F.V.† encephalitis	no
B2	B-N filtrate mouse brain†	0.5	6	2	8	Y.F.V. encephalitis	no
B3	B-V filtrate brain B1	1.0	6	2	8	Y.F.V. encephalitis	no
B4	B-N filtrate brain B2	0.5	7	3	10	Y.F.V. encephalitis	no
B5	B-N filtrate brain B3	1.0	5	2.5	8	Y.F.V. encephalitis	no
B6	B-N filtrate brain B4	0.5	3	2	8	Y.F.V. encephalitis	no
B7	B-N filtrate brain B5	0.5	7	2.5	10	Y.F.V. encephalitis	no
B8	B-N filtrate brain B7	0.5	6	3	9	Y.F.V. encephalitis	no
B9	B-N filtrate brain B8	0.5	2	5.5	9	Y.F.V. encephalitis	no
B10	B-N filtrate brain B8	0.5	3.5	4.5	10	Y.F.V. encephalitis	no
B11	B-N filtrate brain B9	0.5	5	2	7	Y.F.V. encephalitis	no
B12	B-V filtrate brain B10	0.5	12	3	15	Y.F.V. encephalitis	no
B13	B-N filtrate brain B11	0.5	5	2.5	8	Y.F.V. encephalitis	no

TABLE V (continued).

Monkey number.	Inoculum of virus intraspinally.		Febrile reaction.		Final result		
					Death.		Recovery.
	Source.	Amount in c.c.	Days to fever.	Total days of fever.	Days to death.	Cause of death.	
B14	B-N filtrate brain B13*	2.5			9	Y.F.V. encephalitis	no
B15	B-N filtrate brain B14	0.5	6	5	11	Y.F.V. encephalitis	no
B16	B-N filtrate brain B15	1.0	4	4	9	Y.F.V. encephalitis	no
B17	B-N filtrate brain B16	1.0	2	5	7	Y.F.V. encephalitis	no
B18	B-N filtrate brain B17	2.0	3	3.5	7	Y.F.V. encephalitis	no
B19	B-N filtrate brain B18	1.0	1	4.5	7	Y.F.V. encephalitis	no
L25	B-N filtrate brain B18*	2.0	5	2.5	8	Y.F.V. encephalitis	no
L26	B-N filtrate brain L25	1.0	5	6	12	Y.F.V. encephalitis	no
L27	B-N filtrate brain L26	1.0	6	2	9	Y.F.V. encephalitis	no
L28	B-N filtrate brain L27*	0.5	10	1	12	Y.F.V. encephalitis	no

Explanation : * Desiccated.

B-N Berkefeld N filter.

B-V Berkefeld V filter.

† French strain of 148 passages in mice.

‡ Yellow fever virus encephalitis.

passage, a variant of this cultivated strain, grown since the 6th subculture in a medium of mouse embryonic tissue and normal human serum-Tyrode solution has evidenced in mice a constantly shorter average inoculation—death period. Moreover, the human serum strain inoculated both intraspinally and intraperitoneally in two monkeys at the 101st subculture killed both animals of yellow fever virus encephalitis in 10 and 11 days respectively. Each of these

animals received 0.5 c.c. of the culture fluid (titre in mice 100) in the spinal canal and 2.0 c.c. in the peritoneal cavity. Both animals died of yellow fever virus encephalitis, but one presented the additional lesions of confluent lobular pneumonia, and the other showed the concomitant pathology of acute miliary tuberculosis, pulmonary tuberculosis, and tuberculous bronchial lymphadenitis. The two monkeys, bled on the 2nd, 4th and 6th days, showed small amounts of circulating virus, and virus was recovered from the brain of each at death. The significance of these variations in a parallel mouse embryonic tissue strain is important although its interpretation is not clear, and such observations indicate the need of a careful experimental analysis of the degree of neurotropism of any strain of yellow fever virus modified in the laboratory.

In a further effort to test the virulence of the pantropic virus cultivated in mouse embryonic tissue, 0.5 c.c. of the supernatant fluid from the 84th subculture was inoculated intraperitoneally in a hedgehog (*Erinaceus europaeus*), a species demonstrated completely susceptible to yellow fever virus by FINDLAY and CLARKE (1934). The hedgehog, after a short clinical illness of 2 days' duration, characterized chiefly by anorexia and muscular weakness, died 10 days after inoculation with the gross and microscopic lesions of yellow fever in liver, kidney and stomach. The liver showed widespread fatty degeneration and irregular hyaline necrosis, the kidney cloudy swelling, and the stomach profuse haemorrhage from the mucosa. Although the brain revealed no evidence of encephalitis, by ordinary staining methods, virus was recovered from the brain at death.

Although the work of THEILER (1930), PENNA (1935), THEILER and HUGHES (1935), and others has served to demonstrate the original degree of neurotropism present in the pantropic yellow fever virus, the neurotropism of this strain is usually not demonstrable by inoculation extraneurally in man or *Macacus rhesus*. When the pantropic virus is inoculated intracerebrally in mice they die of yellow fever virus encephalitis (THEILER, 1930). If the pantropic virus is inoculated intracerebrally in *M. rhesus*, the monkey dies of yellow fever and the reason usually given is that the monkey dies too quickly of the lesions of yellow fever in viscera other than the brain for encephalitis to progress to fatal issue. However, PENNA (1935) and later FINDLAY and STERN (1935) have shown that if monkeys are inoculated intracerebrally with the pantropic virus, and are at the same time protected against the "viscerotropic" effects of the virus by the simultaneous injection of immune serum they develop encephalitis. Similarly, THEILER and HUGHES (1935) were able to produce fatal encephalitis in the African green monkey (*Lasiopyga callitrichus*) by the intracerebral inoculation of pantropic virus, although members of this species, as shown by BAUER and MAHAFFY (1930), do not become ill of yellow fever when the virus is introduced extraneurally.

Since our experience with the pantropic strain of virus cultivated in mouse embryonic tissue indicated that it was possessed of lesser viscerotropism

and a not greater neurotropism than the original strain, it was considered advisable to passage this strain rapidly through rhesus monkeys in order to determine (1) whether this procedure would bring about an increase of virulence for *M. rhesus* and (2) if an increase of virulence were provoked whether it would manifest itself in an augmentation of its viscerotropic or its neurotropic properties. Accordingly 30 monkeys were inoculated intraperitoneally in series uniformly with 2.0 c.c. of blood or serum. The first animal received 2.0 c.c. of pooled supernatant fluid from the 92nd subcultures of the pantropic virus in mouse embryonic tissue and normal monkey serum-Tyrode solution. The succeeding animals in the series usually received the 3rd day whole blood or serum of the preceding passage monkey. On four occasions the transfer was made with the 2nd day blood or serum and in five instances rehydrated dried serum-containing virus from the preceding passage animal was used as the inoculum. In all other instances direct inoculation of the freshly withdrawn blood was made from monkey to monkey. In Table VI are summarized the essential data of this experiment. Each monkey, if survival permitted, was bled from the saphenous vein on the 2nd, 3rd and 4th days following inoculation. At this time a portion of the separated serum was frozen and desiccated *in vacuo* so that any yellow fever virus in the fluid might be preserved. Correspondingly a group of mice was always inoculated intracerebrally with the serum. Since cultivated strains of yellow fever virus are principally recoverable from the blood stream of monkeys on the 2nd, 3rd, and 4th days following intraperitoneal inoculation, the technique adopted yielded positive information of the course of infection in all animals.

Throughout the series virus was isolated from the blood stream of surviving monkeys on the 2nd, 3rd and 4th days following inoculation. From the beginning to the end of the experiment the virus injected intracerebrally in mice behaved as pantropic yellow fever virus—that is to say, it was characterized by a long inoculation—death period and by a long morbidity period. An examination of Table VI reveals the fact that the first four animals in the series experienced slight, slight, moderate, and no febrile reactions respectively. All four remained apparently well. The 5th and 6th monkeys died of yellow fever, the fifth 7 days and the sixth 5 days after inoculation. The liver parenchymal necrosis was both more intense and more complete in the 6th passage animal. From the 7th to the 13th transfer of the virus no monkey presented fever or clinical illness, although all animals showed circulating virus on the 2nd, 3rd and 4th days following inoculation. The 14th, 16th, 19th, 22nd, 26th, 29th, 30th passage animals suffered fever but recovered, while the 20th, 21st, 25th and 27th monkeys in the series died of yellow fever. Fever occurred in only 10 of the 24 surviving animals. All monkeys, except the six which died of yellow fever were later demonstrated to be immune to the virus. In summary it may be said that the virus behaved for 30 serial passages in monkeys as a pantropic strain of reduced viscerotropic properties. The

TABLE VI.

PATHOGENESIS OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN MOUSE EMBRYONIC TISSUE FOR *M. rhesus* WHEN INOCULATED EXTRANEURALLY FOR SERIAL PASSAGES.

Monkey number.	Passage of virus in monkeys.	Inoculum of virus.†	Circulation of virus mortality ratios in mice inoculated with monkey serum on			Febrile reaction.		Final result.		
								Death.‡	Recovery.	
			Day 2	Day 3	Day 4	Days to fever.	Total days of fever.		Presence of serum anti-bodies.	Response to test inoculation.
W31	1	Fluid of 92nd subcultures	5/6	6/6	5/6	6	0.5	+		nil
W49	2	3rd day blood W31	5/5	6/6	2/4	3	0.5	+		nil
W50	3	3rd day blood W49	5/5	7/7	6/6	4	2.0	+		nil
W51	4	3rd day blood W50	0/3	6/7	5/7		0	+		nil
W52	5	3rd day blood W51	5/6	6/6	6/6	5	1	7Y.F.		
W53	6	3rd day serum W52*	5/6	5/5	6/6	3	1	5Y.F.		
W54	7	3rd day blood W53	4/4	4/6	1/7		0	+		nil
W55	8	2nd day serum W54*	3/5	6/6	5/6		0	+		nil
W56	9	3rd day serum W55*	4/4	4/4	5/7		0	+		nil
W57	10	3rd day blood W56	7/7	4/6	6/6		0	+		nil
W58	11	3rd day blood W57	4/5	5/6	2/6		0	+		nil
W59	12	3rd day blood W58	6/6	6/6	5/6		0	+		nil

TABLE VI (continued).

Monkey number.	Passage of virus in monkeys.	Inoculum of virus.†	Circulation of virus mortality ratios in mice inoculated with monkey serum on			Febrile reaction.		Final result.		
								Death.‡	Recovery.	
			Day 2	Day 3	Day 4	Days to fever.	Total days of fever.		Presence of serum anti-bodies.	Response to test inoculation.
W60	13	3rd day blood W59	3/5	2/4	5/6		0		+	nil
W61	14	2nd day serum W60*	5/6	6/6	5/5	4	2		+	nil
W62	15	3rd day blood W61	6/6	6/6	3/3		0		+	nil
W63	16	3rd day blood W62	5/5	6/6	4/4	3, 12	5		+	nil
W64	17	3rd day blood W63	6/6	5/6	3/4		0		+	nil
W65	18	3rd day blood W64	7/7	5/6	6/6		0		+	nil
W66	19	3rd day blood W65	3/3	5/6	6/6	9	1		+	nil
W67	20	3rd day blood W66	6/6	7/7	7/7		0	4Y.F.		
W68	21	3rd day serum W67*	5/5				0	2Y.F.		
W69	22	2nd day blood W68	6/6	7/7	1/6	2	irregular continued fever		+	nil
W70	23	2nd day blood W69	4/6	3/6	6/6		0		+	nil
W71	24	3rd day blood W70	4/6	5/5	4/4		0		+	nil

TABLE VI (continued).

Monkey number.	Passage of virus in monkeys.	Inoculum of virus.†	Circulation of virus mortality ratios in mice inoculated with monkey serum on			Febrile reaction.		Final result.		
			Day 2	Day 3	Day 4	Days to fever.	Total days of fever.	Death.‡	Recovery.	
									Presence of serum anti-bodies.	Response to test inoculation.
W72	25	3rd day blood W71	3/5	6/6	6/6	3	2	6Y.F.		
W73	26	3rd day blood W72	4/4	6/6	5/6	2, 6	4		+	nil
W99	27	3rd day blood W73	5/5	6/6		2	1.5	4Y.F.		
W100	28	3rd day blood W99	6/6	5/5	3/6		0		+	nil
W101	29	3rd day blood W100	3/4	5/6	4/5	3	1.5		+	nil
W102	30	3rd day blood W101	5/5	5/5	4/4	5, 8	1		+	nil

Explanation : * Rehydrated virus from desiccated state.

— Presence of circulating virus.

† Inoculated uniformly with 2.0 c.c. intraperitoneally.

Y.F. Yellow fever. + Present.

nil No reaction.

‡ Days to death recorded before cause of death.

irregular deaths of the 5th, 6th, 20th, 21st, 25th and 27th passage animals from typical yellow fever are difficult to interpret but may perhaps be best explained as due to a slowly increasing virulence in the pantropic yellow fever virus provoked by rapid passage through the completely susceptible *Macacus rhesus*. With a slowly increasing virulence it might be anticipated that great variations in its apparent effects in susceptible monkeys would be observed. While a highly virulent strain might kill all monkeys and a strain of low virulence no monkeys, it is to be expected that with strains of intermediate virulence, the more susceptible animals would succumb to the disease, the less susceptible would exhibit fever or slight illness, and the least susceptible would remain clinically well. The behaviour of the virus in its later passages through monkeys

very closely parallels the behaviour of the same virus in *M. rhesus* during its earlier subcultures in mouse embryonic tissue (cf. passages 20 to 30 in Table VI with subcultures 15 to 25 in Table III, p. 498). It is a significant fact, worthy of emphasis, that the only deaths provoked by the virus in monkeys were due to yellow fever and not to yellow fever virus encephalitis. This is good evidence of the production of a strain of lesser pantropic virulence without increase of neurotropic virulence. Correlative evidence on this point has been obtained by THEILER (1935) who has been able to demonstrate that similar extraneural serial passage by blood transfer of the neurotropic virus through 20 passages in *M. rhesus*, although it at no time produced yellow fever in the experimental animal, killed by yellow fever virus encephalitis 5 of 16 monkeys in which the infection was allowed to run its full course. Thirteen of these 16 animals showed fever of an average duration of 3·8 days.

PATHOGENESIS OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN CHICKEN EMBRYONIC TISSUE.

The strain of pantropic yellow fever virus cultivated in chicken embryo tissue originated from the parent mouse embryonic virus after 18 subcultures in mouse embryonic tissue. The pathogenesis of this strain for mice is summarized in the results recorded in Table I (p. 491). Excepting the first 10 passage average which is based on a period in which the virus was inoculated in mice after 7 days' incubation instead of the usual 3 to 4 day interval, the average inoculation—death period for these animals showed no significant increase or decrease during the course of the cultivation experiment. On the other hand, the virulence of the strain for mice following intracerebral inoculation corresponded closely both with the other cultivated strains of pantropic virus and with the parent pantropic strain of monkey origin. A strain of pantropic virus grown for more than 30 subcultures in chicken embryo dermis did not show during this time an appreciably different pathogenesis for mice from that of the virus in whole chicken embryo tissue.

The pathogenesis of the chicken embryo strain of pantropic virus for *M. rhesus* was tested on only one occasion. The pooled supernatant fluid from the 89th subculture containing virus of a titre of 1000 for mice, was inoculated into 9 monkeys. Each animal showed an absence of protecting antibodies in its serum at the time of injection. Three of the monkeys received 0·5 c.c. of the culture fluid in the spinal canal; three others received 0·5 c.c. of the culture fluid intraspinally as well as 2·0 c.c. intraperitoneally; finally three animals were inoculated with 2·0 c.c. of the culture fluid in the peritoneal cavity. Each monkey in the series was bled from the vein on alternate days from the 2nd to the 7th and virus was demonstrated in the circulating blood of each animal for the greater part or all of this period. All monkeys with the exception of one inoculated both intraperitoneally and intraspinally, and two of the three inoculated intraperitoneally suffered febrile

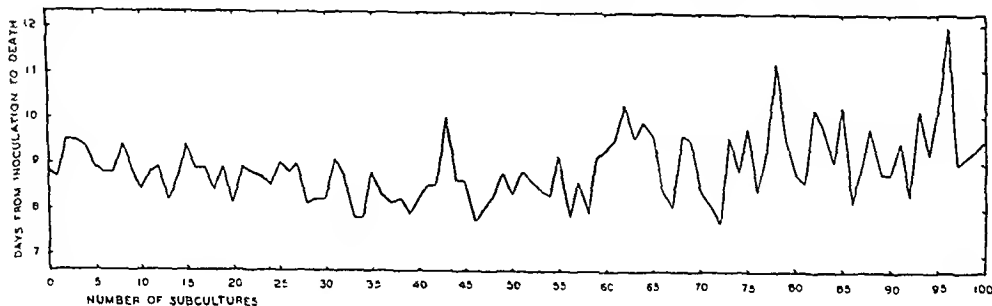
attacks commencing 3 to 6 days after inoculation and lasting for 2 to 6 days. Each monkey which received virus in the spinal canal died in 10 to 14 days of yellow fever virus encephalitis proven by the clinical symptomatology, the isolation of the virus from the brain at death and the demonstration of the histological lesions of the disease. One of the three animals inoculated intraperitoneally suffered fever from the 3rd to the 7th day and died on the 16th day after inoculation with the lesions of general miliary tuberculosis, recovering yellow fever and yellow fever virus encephalitis. Although this animal showed no signs of encephalitis during life, virus was recovered from the brain at death and microscopical examination of the nervous system revealed scattered ganglion cell degeneration. The serum of this monkey showed protecting antibodies to a titre of 13 at the time of death. The two monkeys which survived intraperitoneal inoculation of the virus were demonstrated after 24 days to have serum antibody titres of 10 and 64 respectively. They were also shown to be immune to test inoculation with virulent pantropic virus.

PATHOGENESIS OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN MOUSE TESTICULAR TISSUE.

The strain of yellow fever virus grown in adult mouse testicular tissue and normal human serum-Tyrode originated from the parent mouse embryonic strain after 27 subcultures in mouse embryonic tissue. The pathogenesis of this strain for mice is summarized in the results recorded in Table I. A more detailed record of the average inoculation-death periods for each subculture for 100 passages in this medium is shown in Graph 2. The 0 subculture recorded in the Graph is the 28th passage of pantropic virus in flask cultivation and for purposes of comparison the latter method of recording is used in Table I. Although the virus showed no significant alteration in the degree of neurotropism for mice as measured by the inoculation-death period during the first 60 subcultures (87 subcultures in Table II) a critical examination of both Table I and Graph 2 will demonstrate that in the latter 40 passages the existing trend is towards a prolongation of this period.

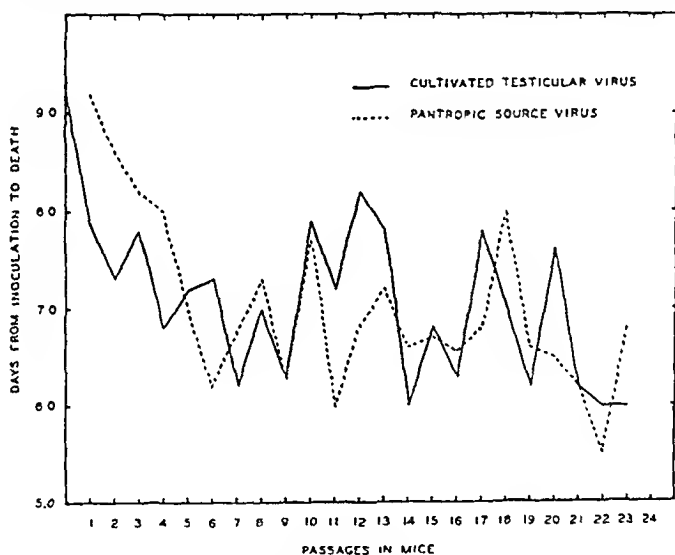
At the time of the 42nd subculture of the virus in mouse testicular tissue

GRAPH 2.—NEUROTROPISM FOR MICE OF VIRUS CULTIVATED IN TESTICULAR TISSUE.



and the 69th passage of flask cultivation the degree of neurotropism of the cultivated virus was compared with that of the pantropic virus of Asibi strain used as source for the cultivation experiments. An exact comparison was possible because the original source virus of monkey passage was available through preservation in the dried state. The cultivated strain and the original were passaged regularly through the same strain of mice for twenty-three transfers. This is a method designed to show any difference in degree of fixation for the nervous tissues of mice between the strains compared. From Graph 3 the results of this experiment may be readily interpreted, the number of mouse passages having been plotted against the time in days from inoculation to death. Recognizing the considerable variation in the length of the inoculation-

GRAPH 3.—COMPARATIVE RATE OF FIXATION IN MOUSE BRAIN OF CULTIVATED TESTICULAR VIRUS AND THE ORIGINAL PANTROPIC VIRUS.



death period from passage to passage due to uncontrollable differences in the amount of virus in the brain used for transfer, the two curves are remarkably similar. Both show an initial sudden fall, occasioned by a rapid lessening of the inoculation-death period during the earlier transfers followed by a slower but consistently progressive decrease in the length of the period with later passages. The slower but progressive fixation occurring after the first few transfers is somewhat obscured in the Graph by the wide variations from passage to passage but it is nevertheless evident. The detail in Graph 3 offers weighty evidence to show that the degree of neurotropism for mice of the original pantropic virus of monkey origin and the cultivated strain was approximately equal at the time of the 69th subculture *in vitro*.

During three different periods of the cultivation of pantropic virus in mouse testicular tissue, experiments were carried out to test the susceptibility of *M. rhesus* to the virus when inoculated by various routes. The results of these experiments are brought together in Table VII.

PATHOGENESIS OF ANTROPIC YELLOW FEVER VIRUS

Monkey number.	Inoculum of virus.				Induced cerebral injury.†	Circulation of virus. Serum inoculated in groups of mice. Mortality ratios in mice on following days.					
	Fluid of subculture.	Amount of fluid. in c.c.	Quantity of virus.	Route.		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
W74	30	0.5		I.S.							
W75	30	0.5		I.P.							
W76	30	2.0		I.P.							
W77	30	2.0		I.P.							
W78	35	0.5		I.S.			<u>2/5</u>		<u>3/6</u>		0/5
W79	35	0.5		I.S.			<u>2/7</u>		0/6		0/4
W80	35	0.5		I.S.			0/6		0/6		1/6
W81	35	2.0		I.P.			0/6		0/4		0/4
W82	35	2.0		I.P.			0/6		0/6		0/6
W83	35	2.0		I.P.			0/5		<u>1/6</u>		0/5
W84	43	10.0	334,000 MLD.	I.V.	+	<u>4/4*</u>	<u>6/6*</u>	<u>6/6*</u>	<u>2/5*</u>	<u>5/6*</u>	0/3
W85	43	10.0	334,000 MLD.	I.V.	+	<u>3/6*</u>	<u>6/6*</u>	<u>6/6*</u>	<u>2/5*</u>	<u>1/5*</u>	0/3
W86	43	0.5	17,000 MLD.	I.D.	+	<u>0/5*</u>	<u>0/5*</u>	<u>0/4*</u>	<u>0/5*</u>	<u>0/6*</u>	0/2
W87	43	0.5	17,000 MLD.	I.D.	+	<u>0/4*</u>	<u>0/6*</u>	<u>1/5*</u>	<u>5/6*</u>	<u>5/5*</u>	3/4
W88†	Neurotropic virus F109	0.5	1,700,000 MLD.	I.D.	+	<u>2/4*</u>	<u>5/5*</u>	<u>6/6*</u>	<u>6/6*</u>	<u>5/5*</u>	5/5

Explanation : — Presence of circulating virus.
 Y.F.V. Yellow fever virus.
 * Serum also titrated for virus in dilutions of 1-1,000 and 1-1,000,000 but these dilutions produced no morbidity in mice.

† 1.0 c.c. monkey's own blood injected intracerebrally.
 + Presence of cerebral injury.
 MLD. Minimal lethal dose for a mouse.
 T Titre.

VII.

CULTIVATED IN MOUSE TESTICULAR TISSUE FOR *M. rhesus*.

Circulation of virus. Serum inoculated in groups of mice. Mortality ratios in mice on following days.					Febrile reaction.		Final result.			
							Death.		Survival.	
Day 7	Day 8	Day 9	Day 11	Day 13	Days to fever.	Total days of fever.	Days to death.	Cause of death.	Presence of serum antibodies.	Response to test inoculation
					8	1	10	Y.F.V. encephalitis		
						0			T11	nil
					9	6			T48	nil
						0			T1	nil
	0/6				4, 8	2	13	Y.F.V. encephalitis		
	0/5				4	5	11	Y.F.V. encephalitis		
	0/6				4	5	10	Y.F.V. encephalitis		
	0/6					0			T13	nil
	0/5					Irregular fevers Tuberculosis			T16	
	0/7					0			T1.5	nil
0/5*	0/4*	0/5*	0/6*		6	5			T64	nil
0/5*	0/6*				6	3	10	Y.F.V. encephalitis		
0/6*	0/5*	0/5*	0/5*	0/6*		0			T50	nil
2/5*	2/5*	5/6*	0/6*		9, 15	1			T64	nil
0/5*	0/6*	0/5*			2	7.5	12	Y.F.V. encephalitis		

† Control inoculated with neurotropic yellow fever virus of the French strain and of 109 passages in mice.
nil No reaction.

I.S. Intraspinal.
I.V. Intravenous.
I.P. Intraperitoneal.
I.D. Intradermal.

One monkey was inoculated intraspinally and three intraperitoneally with the virus of the 30th subculture. The former animal died of yellow fever virus encephalitis while the latter three were immunized, and in two of the three instances without the occurrence of fever.

At the time of the 35th subculture, 3 monkeys were injected with the culture fluid in the spinal canal and three others received the virus in the peritoneal cavity. All three animals inoculated intraspinally died of encephalitis while the three monkeys inoculated intraperitoneally were immunized without specific febrile reactions. The minimal amount of circulating virus recovered from the blood streams of all six of these animals is indicated in Table VII.

Two monkeys were injected intravenously and two intradermally with the testicular culture virus of the 43rd subculture. At the same time, as a control on the inoculations, an additional monkey was inoculated in the skin with neurotropic yellow fever virus of 109 passages in mice. Immediately preceding inoculation each of the five animals was injected intracerebrally with 1.0 c.c. of its own freshly drawn defibrinated blood in order to produce a moderately severe cerebral lesion. SAWYER and LLOYD (1931) had previously demonstrated the importance of cerebral injury in mice in localizing circulating virus in the brain. The occurrence of cerebral vascular lesions or permeable haematocephalic barrier has in latter years been considered to offer a serious risk in immunization against yellow fever in the presence of circulating virus. Of the five monkeys used in this experiment, only one inoculated intradermally with the culture virus failed to show circulating virus during the 13-day period of daily blood infectivity tests. This animal was later shown to be highly immune as were two others receiving the same inoculum intradermally and intravenously respectively. One monkey injected intravenously with the cultivated testicular virus and intracerebrally with blood developed encephalitis and succumbed as did a control animal inoculated intradermally with a fixed neurotropic strain of the virus.

PATHOGENESIS OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN GUINEAPIG TESTICULAR TISSUE.

The strains of virus cultivated in guineapig testicular tissue originated from the strain grown in mouse testicular tissue after 41 and 70 subcultures respectively in the latter medium. In both instances the virus grown in guineapig tissue showed an inoculation-death period appreciably longer on the average than that of other pantropic strains. However, the virus was not cultivated long enough in this medium for the conclusion to be certain. The inoculation-death periods for the 10 passage averages were 9.4 and 9.8 days in the first series and 9.5, 9.2 and 9.4 days in the second series. The pathogenesis of this strain for *M. rhesus* was tested on only one occasion. The virus of the 17th subculture of the first series inoculated intraspinally produced fatal encephalitis in 11 days.

PATHOGENESIS OF CULTIVATED NEUROTROPIC YELLOW FEVER VIRUS.

Although in these experiments the pathogenesis of the three neurotropic strains for *M. rhusus* was never tested, HAAGEN (1933) found that a strain of neurotropic yellow fever virus, grown for more than 100 passages in chicken embryo tissue in Carrel flasks, provoked a fatal encephalitis when inoculated intracerebrally in monkeys.

The degree of neurotropism for mice of the cultivated neurotropic strains is well indicated in Table I. In the Table the average inoculation-death periods for each 10 subcultures are summarized. The strain of French neurotropic virus cultivated in mouse embryonic tissue after 305 passages in mice showed an average inoculation-death period 2 days longer than that of the original mouse passage virus. The strain of the same origin grown in chicken embryo tissue presented an average period 2.5 days longer than that of the mouse passage strain. The French neurotropic virus grown in chicken embryo tissue, after 108 mouse passages showed consistently an average inoculation-death period 1 to 1.5 days longer than those of the cultivated neurotropic strains of 305 mouse passages. This period was 3 days longer than the average of the original virus of 108 mouse passages. These data clearly demonstrate two conditions (1) the neurotropic strains are characterized early in the period of their cultivation and consistently thereafter by an appreciably longer inoculation-death period in mice than those evinced by the parent mouse passage viruses, (2) the degree of neurotropism or fixation for mouse brain is reflected in the behaviour of the neurotropic viruses during the period of cultivation. The latter conclusion is based on the fact of the longer inoculation—death periods of the less fixed virus of 108 mouse passages than those of the more fixed viruses from the same original strain of 305 mouse passages. A final survey of Table I (p. 491) will also serve to demonstrate the appreciable differences in the degree of neurotropism under similar conditions of cultivation of the originally pantropic strains of yellow fever virus and the neurotropic strains.

THE ACTIVE IMMUNIZATION OF MONKEYS WITH PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN MOUSE EMBRYONIC TISSUE AND COMPLEMENTARY IMMUNE SERUM.

In Tables VIII and IX, are summarized the results of two complementary experiments. In the first, four monkeys were inoculated subcutaneously with a constant quantity of virus 1.0 c.c. (1.0 c.c. = 100,000 minimal lethal doses for a mouse) and an amount of human immune serum (titre 128) varying from 0.5 to 0.05 c.c. per kilogram of body weight. A fifth monkey received an equal dose of virus subcutaneously without complementary immune serum. In the second experiment four monkeys were inoculated subcutaneously with a constant amount of human immune serum (titre 128)

PATHOGENICITY FOR *M. rhesus* OF A FIXED QUANTITY OF CULTIVATED

Monkey number.	Monkey weight.	Inoculum of virus.*	Inoculum of serum.*		Circulation of virus. Serum inoculated in groups of mice. Mortality ratios groups of mice on following days.						
			Amount in c.c. per kilogram.	Total amount in c.c.	Titre.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
W89	2,350 gm.	100,000 MLD.	0.5	1.2	128	0.5	0.4	0/6	0/5	0/5	0/6
W90	2,050 gm.	100,000 MLD.	0.2	0.4	128	0.5	0/6	0/6	3/4	4/6	6/6
W91	2,000 gm.	100,000 MLD.	0.1	0.2	128	0.4	0.4	2/5	3/5	4/5	2/5
W92	2,000 gm.	100,000 MLD.	0.05	0.1	128	0.5	1/4	2/6	5/5	6/6	2/5
W93	2,300 gm.	100,000 MLD.				0.4	4/5	5/5	5/5	6/6	6/6

Explanation : * Virus and serum were both injected subcutaneously.
MLD. Minimal lethal dose for a mouse.

in a ratio of 0.5 c.c. per kilogram of body weight and a quantity of virus varying from 100,000 to 100 minimal lethal doses for a mouse. A fifth monkey in the second series received subcutaneously human immune serum in the ratio of 5.0 c.c. per kilogram of body weight and a quantity of virus amounting to 34 minimal lethal doses for a mouse. An absence of serum protecting antibodies was demonstrated in all monkeys at the time of inoculation. Mice inoculated to demonstrate the presence of circulating virus were observed for a period of 21 days. The virus used in both experiments was the same and consisted of a constant source of dried virus prepared from the S2nd and S3rd subcultures of the pantropic virus in mouse embryonic tissue, by centrifuging out the cells, diluting the supernatant fluid with an equal quantity of a known normal human serum and desiccating the resulting fluid in the frozen state in vacuo. These virus preparations were titrated in mice at frequent intervals to determine their constancy and at the time of use in each experiment. The titre of the virus was uniformly taken as the highest dilution of the preparation which in 0.03 c.c.

VIII.

PANTROPIC VIRUS WITH VARYING AMOUNTS OF IMMUNE SERUM.

Circulation of virus. Serum inoculation in groups of mice. Mortality ratios in groups of mice on following days.								Febrile reaction.		Final result. Presence of serum antibodies after inoculation.			
Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Days to fever.	Total days of fever.	14 days.		28 days.	
										Prescnce.	Titre.	Presence.	Titre.
0/4	0/6	0/5	0/4	0/5	0/6	0/5	0/5		0	+		+	154
3/6	0/5	0/6	0/5	0/4	0/4	0/5	0/6		0	+		+	256
0/5	0/6	0/5	0/5	0/4	0/5	0/6	0/4	13	4	+		+	218
4/6	0/6	0/5	0/5	0/6	0/3	0/4	0/4		0	+		+	173
1/3	0/6	0/6	0/5					3, 7	1	+	+256	+	32

— Presence of circulating virus.
+ Present.

amounts for each mouse inoculated intracerebrally would kill half of the mice injected, and in practice this amount was mathematically calculated according to the method of MUENCH. The mice were inoculated with each dilution in groups of either 6 or 12. Thus 1.0 c.c. of a virus preparation which killed half the mice injected intracerebrally with 0.03 c.c. amounts in a dilution of 1/3,300 would contain by these standards approximately 100,000 minimal lethal doses for a mouse. The titre of the serum was taken as the highest dilution of the immune serum (diluted in 10 per cent. normal serum-saline solution) which would protect from death of yellow fever virus encephalitis half of the mice inoculated with the virus-serum dilution (intraperitoneal protection test in mice, SAWYER and LLOYD, 1931). All titrations of protecting antibodies at different periods following the inoculation of the several monkeys were made against the same virus in each series except in the case of the 14 day titration of the serum of monkey W93 and the weekly sera of monkey W98 which were made against virus of a different source from the others of the series.

PATHOGENICITY FOR *M. rhesus* OF A FIXED QUANTITY OF CULTIVATED

Monkey number.	Monkey weight.	Inoculum of virus.*	Inoculum of serum.*			Circulation of virus. Serum inoculated in groups of mice. Mortality ratios groups of mice on following days.					
			Amount in c.c. per kilogram.	Total amount in c.c.	Titre.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
W89	2,350 gm.	100,000 MLD.	0.5	1.2	128	0/5	0/4	0/6	0/5	0/5	0/6
W90	2,050 gm.	100,000 MLD.	0.2	0.4	128	0/5	0/6	0/6	3/4	4/6	6/6
W91	2,000 gm.	100,000 MLD.	0.1	0.2	128	0/4	0/4	2/5	3/5	4/5	2/5
W92	2,000 gm.	100,000 MLD.	0.05	0.1	128	0/5	1/4	2/6	5/5	6/6	2/5
W93	2,300 gm.	100,000 MLD.				0/4	4/5	5/5	5/5	6/6	6/6

Explanation : * Virus and serum were both injected subcutaneously.
MLD. Minimal lethal dose for a mouse.

in a ratio of 0.5 c.c. per kilogram of body weight and a quantity of virus varying from 100,000 to 100 minimal lethal doses for a mouse. A fifth monkey in the second series received subcutaneously human immune serum in the ratio of 5.0 c.c. per kilogram of body weight and a quantity of virus amounting to 34 minimal lethal doses for a mouse. An absence of serum protecting antibodies was demonstrated in all monkeys at the time of inoculation. Mice inoculated to demonstrate the presence of circulating virus were observed for a period of 21 days. The virus used in both experiments was the same and consisted of a constant source of dried virus prepared from the 82nd and 83rd subcultures of the pantropic virus in mouse embryonic tissue, by centrifuging out the cells, diluting the supernatant fluid with an equal quantity of a known normal human serum and desiccating the resulting fluid in the frozen state in vacuo. These virus preparations were titrated in mice at frequent intervals to determine their constancy and at the time of use in each experiment. The titre of the virus was uniformly taken as the highest dilution of the preparation which in 0.03 c.c.

VIII.

PANTROPIC VIRUS WITH VARYING AMOUNTS OF IMMUNE SERUM.

Circulation of virus. Serum inoculation in groups of mice. Mortality ratios in groups of mice on following days.								Febrile reaction.		Final result. Presence of serum antibodies after inoculation.			
								Days to fever.	Total days of fever.	14 days.		28 days.	
Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14			Presence.	Titre.	Presence.	Titre.
0/4	0/6	0/5	0/4	0/5	0/6	0/5	0/5		0	+		+	154
<u>3/6</u>	0/5	0/6	0/5	0/4	0/4	0/5	0/6		0	+		+	256
0/5	0/6	0/5	0/5	0/4	0/5	0/6	0/4	13	4	+		+	218
<u>4/6</u>	0/6	0/5	0/5	0/6	0/3	0/4	0/4		0	+		+	173
<u>1/3</u>	0/6	0/6	0/5					3, 7	1	+	+256	+	32

— Presence of circulating virus.

+ Present.

amounts for each mouse inoculated intracerebrally would kill half of the mice injected, and in practice this amount was mathematically calculated according to the method of MUENCH. The mice were inoculated with each dilution in groups of either 6 or 12. Thus 1.0 c.c. of a virus preparation which killed half the mice injected intracerebrally with 0.03 c.c. amounts in a dilution of 1/3,300 would contain by these standards approximately 100,000 minimal lethal doses for a mouse. The titre of the serum was taken as the highest dilution of the immune serum (diluted in 10 per cent. normal serum-saline solution) which would protect from death of yellow fever virus encephalitis half of the mice inoculated with the virus-serum dilution (intraperitoneal protection test in mice, SAWYER and LLOYD, 1931). All titrations of protecting antibodies at different periods following the inoculation of the several monkeys were made against the same virus in each series except in the case of the 14 day titration of the serum of monkey W93 and the weekly sera of monkey W98 which were made against virus of a different source from the others of the series.

PATHOGENICITY FOR *M. rhesus* OF A FIXED AMOUNT OF IMMUNE

Monkey number.	Monkey weight.	Inoculum of virus.*	Inoculum of serum.*			Circulation of virus.					
			Amount in c.c. per kilogram.	Total amount in c.c.	Titre.	Serum inoculated in groups of mice. Mortality ratios groups of mice on following days.					
						Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
W94	2,000 gm.	100,000 MLD.	0.5	1.0	128	0/2	0/5	0/6	0/4	0/5	0/6
W95	1,850 gm.	10,000 MLD.	0.5	0.9	128	0/5	0/5	0/5	0/6	0/6	0/5
W96	2,000 gm.	1,000 MLD.	0.5	1.0	128	0/5	0/5	0/6	<u>3/6</u>	<u>3/6</u>	<u>3/6</u>
W97	1,850 gm.	100 MLD.	0.5	0.9	128	0/4	0/6	0/5	<u>1/6†</u>	0/6	<u>1/6†</u>
W98	2,200 gm.	34 MLD.	5.0	11.0	128	0/6	0/7	0/6	0/6	0/5	0/6

Explanation: * Virus and serum were both injected subcutaneously.

MLD. Minimal lethal dose for a mouse.

 Presence of circulating virus.

Since each of these animals was later added to its respective series for supplementary comparison the inconsistency is not important.

The results of the experiment summarized in Table VIII clearly show that at least 0.5 c.c. per kilogram of body weight of a human immune serum having a titre of 128 is necessary to prevent the demonstration of circulating virus in the blood of *Macacus rhesus* during the period from inoculation to the appearance of serum protecting antibodies, when the inoculating dose equals 100,000 minimal lethal doses for a mouse. Reference to Table IX will show that even this degree of passive immunity is not always sufficient to prevent the demonstration of virus in the blood. The results in Table IX also demonstrate that active immunity develops in the monkey in the presence of a high or of an extreme degree of passive immunity even though the virus inoculum is reduced to very small quantities. The degree of active immunity as evidenced by circulating serum antibodies was, however, suggestively less following the inoculation of the smaller quantities of virus.

IX.

SERUM WITH VARYING QUANTITIES OF CULTIVATED PANTROPIC VIRUS.

Circulation of virus.								Febrile reaction.	Final Result.				
Serum inoculated in groups of mice. Mortality ratios groups of mice on following days.									Serum antibody titre after inoculation.			Response to test inoculation.	
Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Days to fever.	Total days of fever.	14 days.	21 days.		28 days.
0/4	0/5	0/2	0/3	0/4	0/4	0/5	0/5		0	48	115	109	—
0/6	0/6	0/6	0/5	0/5	0/5	0/5	0/6		0	30	20	16	nil
0/6	0/6	0/5	0/4	0/5	0/5	0/4	0/6		0	33	42	30	nil
0/5	0/5	0/6	0/5	0/5	0/5	0/5	0/6		0	12	12	11	nil
0/5	0/5	0/5	0/6	0/6	0/2	0/5	0/6		0	+	+	42 days 4	nil

† Death from yellow fever virus encephalitis proven.

+ Complete protection of mice by undiluted serum at 14, 21 and also at 28 days.

nil No reaction.

THE ACTIVE IMMUNIZATION OF MAN WITH PANTROPIC YELLOW FEVER VIRUS
CULTIVATED IN MOUSE EMBRYONIC TISSUE AND COMPLEMENTARY IMMUNE
SERUM.

Materials and Methods.

Cultivated virus used for the active immunization of man against yellow fever was always prepared in the following way : 24 hours before the preparation of a batch of virus for human immunization bacterial broth cultures were made from each flask of cultivated virus by the addition of 0.1 c.c. of culture fluid to the broth tube. On the following day the culture fluid from a series of bacteriologically sterile flasks containing the virus growing in a medium of mouse embryonic tissue and normal human serum-Tyrode solution was pooled and centrifuged for 10 minutes at 3,000 r.p.m. The clear supernatant fluid after centrifugalization was pipetted off and mixed with an equal quantity of filtered normal human serum. The mixture was placed in 0.5 c.c. quantities in

Wassermann tubes. The contents of these tubes were frozen in alcohol chilled with solid carbon dioxide and desiccated in vacuo over sulphuric acid while still in the solid state in desiccators of the Hempel improved type. Aerobic and anaerobic broth cultures as well as blood agar slant cultures were made on the culture fluid, the serum diluent and the mixture of both fluids, and were demonstrably sterile before use of the virus.

The virus content of such preparations was determined by taking random samples from each desiccator, rehydrating the dried powder with 0.5 c.c. distilled water, and inoculating intracerebrally into groups of six mice serial decimal dilutions of 1/10 to 1/100,000. All dilutions were made in 10 per cent. normal human serum-saline solution. The titre of the virus was taken as the highest dilution which would effect a 50 per cent. mouse mortality when the mortality ratios were subjected to the calculation method of MUENCH.

In Table X the average virus titres of 15 consecutive lots of dried virus used for human immunization are compared with the virus titres of the same lots before desiccation. Excepting the first two lots of virus which were prepared in the early spring, the others were desiccated during the hot humid months of summer in New York, and the fall in virus content from the fresh to the desiccated state is correspondingly much greater. Nevertheless, each virus sample contained an adequate immunizing dose for a passively immunized man. In this long series in which random ampoules from each desiccator were titrated for virus content not one was found containing less than 1,700 minimal lethal doses of virus for a mouse. Equally important, no culture flask, each of which was carefully controlled bacteriologically, was found to be contaminated during a long period in which 2,000 tubes of desiccated cultivated virus were prepared.

Immune human serum was harvested from the blood of men who had recovered from yellow fever or possessed serum protecting antibodies as a result of vaccination against the disease. Sera from several individuals were pooled and filtered. The serum protecting antibody content was determined by titration in mice by the technique of the intraperitoneal protection test (SAWYER and LLOYD, 1931). The titre of serum was taken as the highest dilution which would protect half of the mice inoculated from death of yellow fever virus encephalitis. Mortality ratios were summed and the dilution which would afford a 50 per cent. protection calculated according to the method of MUENCH.

Sera having an antibody titre by mouse test of 100, if previously administered in a ratio of 0.5 c.c. per kilogram of body weight would ordinarily protect *rhesus* monkeys from fever or illness after injection of fully virulent pantropic virus of the Asibi strain. An equal or slightly greater antibody content in the serum protected monkeys against the demonstration of circulating virus. Only sera titrated for protection of monkeys by these criteria or sera standardized against known immune sera by protecting antibody titrations in mice were used in human immunization experiments.

TABLE X.

COMPARISON OF THE VIRUS TITRE OF PANTROPIC YELLOW FEVER VIRUS CULTIVATED IN MOUSE EMBRYONIC TISSUE BEFORE AND AFTER DESICCATION IN THE SOLID STATE.

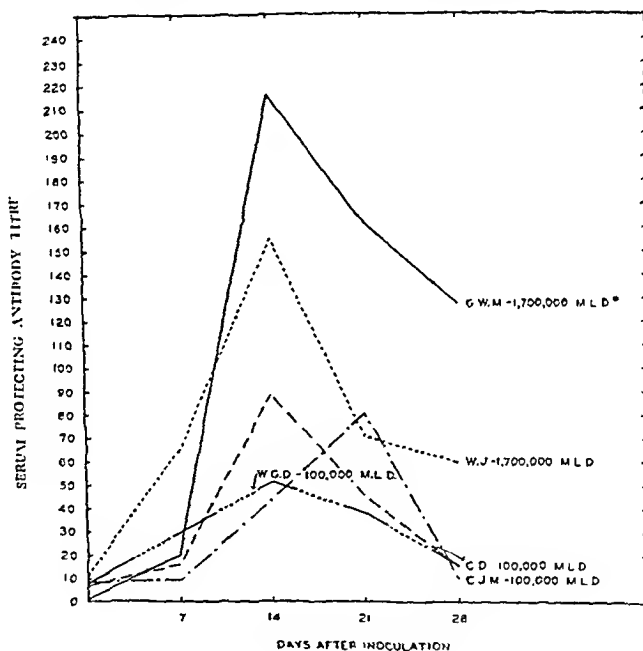
Number of subculture.	Titre of virus in supernatant culture fluid.	Estimated titre after mixture with equal parts of normal serum.	Titre after desiccation in the solid state.
82	1,000	500	485
83	13,500	6,750	6,100
112	7,300	3,650	550
113	17,500	8,750	2,400
114	20,000	10,000	415
115	7,000	3,500	1,500
116	13,000	6,500	135
117	4,000	2,000	410
118	10,000	5,000	410
119	10,000	5,000	1,900
120	7,100	3,550	820
121	1,000	500	450
122	2,000	1,000	1,200
123			1,900
124	4,400	2,200	2,200

ANTIGENICITY FOR MAN OF THE CULTIVATED PANTROPIC VIRUS OF MOUSE EMBRYONIC TISSUE ORIGIN.

An attempt to compare the antigenicity for immune man of the pantropic strain of yellow fever virus cultivated in mouse embryonic tissue with that of the neurotropic virus of mouse brain origin is summarized in Table XI. In Table XI approximately equal results in the degree of titrable serum immunity produced have purposely been grouped consecutively for comparison. Careful study of the Table will show that irrespective of whether the virus inoculated

into immune man was the cultivated strain or the mouse passage virus, the degree of antigenic response as indicated by the multiplication and decline of titre during a 28 day period of observation was closely similar. Following the inoculation of both strains of yellow fever virus the highest titre was observed at the 14 day period followed by a decreasing titre thereafter. There was but one exception to this rule when in the case of C.J.M. a higher titre was recorded on the 21st day than on the 14th day. In Graph 4, the curves of titrable antibodies for weekly intervals on five immune men are recorded. In this Graph the rise and fall in antibody content following the new introduction of antigen

GRAPH 4.—SERUM PROTECTING ANTIBODY TITRE IN IMMUNE MAN FOLLOWING A SECOND INOCULATION OF YELLOW FEVER VIRUS.



* Minimal lethal dose for a mouse

is well demonstrated. The suggestion is also clear that a greater antibody response follows the inoculation of a larger amount of virus in immune man. The virus titres noted in Table XI were resolved by applying the method of MUENCH directly to the mortality ratios of groups of mice inoculated with the several dilutions of the virus-containing fluid in order to obtain the dilution productive of a 50 per cent. mortality. Similarly the calculated titre of serum protecting antibodies was obtained by applying the method of MUENCH to the protection ratios of groups of mice inoculated with the several serum dilutions and a constant amount of virus according to the technique of the intraperitoneal

protection test in mice (SAWYER and LLOYD, 1931). Since in this test the quantity of virus and hence the amount of titrable antibody in a constant serum varies from one series of tests to another, the calculated antibody titre for each serum sample was corrected against the titre of a known immune serum. Thus if in a given series of titrations a constant immune serum, known to have an average titre of 100, showed a calculated titre of 80, the antibody contents of all immune sera titrated in the same series were corrected as $\frac{100}{80}$ of the calculated titre. This revised titre is recorded in Table XI (p. 524) as the corrected titre.

IMMUNIZATION OF MAN WITH A KNOWN QUANTITY OF VIRUS AND A KNOWN QUANTITY OF IMMUNE SERUM.

The results of the immunization of 26 men with a known quantity of the virus cultivated in mouse embryonic tissue and a known amount of human immune serum according to the method described by SAWYER, KITCHEN and LLOYD (1932) are recorded in Table XII. For the immunization of seven persons in this series and for subsequent studies on these cases the writers are indebted to the kindness of Dr. G. M. FINDLAY of London, and Dr. W. A. SAWYER of New York. The immunized human subjects received an amount of virus varying between 14,000 and 170,000 minimal lethal doses for a mouse together with a quantity of human immune serum varying between 0.5 c.e. and 0.6 c.e. per kilogram of body weight. The titre of protecting antibodies in the immune serum employed varied between 94 and 256. Of the 26 persons inoculated only three developed fever of 37.7° C. Five others showed a rise of 0.5° C. or less, while 18 presented no elevation of temperature. One man complained of headache, sensation of cold and malaise, on the evening of the third day following inoculation. None of the others exhibited any signs of illness during the period of observation. In only two of the inoculated men were detailed observations possible. Each of these showed leucopenia (4,000 leucocytes per c.mm.), one on the 5th, 6th and 7th days, and the other on the 6th day. Neither exhibited albuminuria at any time. For 13 persons the amount of titrable protecting serum antibody was investigated during the period from 14 to 28 days following inoculation. Each individual thus tested showed the presence of protecting antibodies, the titre of which varied between 8 and +256. All of five immunized persons so studied showed no circulating virus following inoculation. The reactions following inoculation were minimal or absent. It is however worthy of note that slight febrile reactions when they were observed occurred in a period 2 to 6 days following inoculation and most often on the evening of the 3rd or 4th day. This is the incubation period of yellow fever. The febrile reaction of yellow fever virus encephalitis is later, more marked and more prolonged, occurring from 7 to 14 days following inoculation.

TABLE XI.

COMPARISON OF THE ANTIGENICITY FOR MAN OF THE NEUROTROPIC VIRUS OF MOUSE BRAIN ORIGIN, AND THE CULTIVATED PANTROPIC VIRUS OF MOUSE EMBRYONIC TISSUE ORIGIN.

Designation of man.	Inoculum of virus.		Serum protecting antibody titre.									
			Before inoculation.	After inoculation.								
				7 days.		14 days.		21 days.		28 days.		
	Mouse neurotropic virus.	Cultivated pantropic virus.	Calculated titre.	Corrected titre.	Calculated titre.	Corrected titre.	Calculated titre.	Corrected titre.	Calculated titre.	Corrected titre.	Calculated titre.	Corrected titre.
G.W.M.	1,700,000 MLD.		(1)	1	(40)	20	(128)	216	(96)	162	(154)	128
W.K.S.-T.		100,000 MLD.	(5)	5							(154)	154
H.L.F.	1,700,000 MLD.		(11)	11							(55)	28
S.C.		100,000 MLD.	(6)	6							(27)	14
W.J.	1,700,000 MLD.		(11)	11	(128)	65	(92)	155	(42)	71	(118)	60

N.I.R.		100,000 MLD.	(6)								(42)	42
T.P.H.	100,000 MLD.		(22)	22							(36)	18
W.A.S.		100,000 MLD.	(38)	130							(46)	78
L.M.M.	100,000 MLD.		(112)	156							(27)	27
J.H.B.		100,000 MLD.	(14)	14							(8)	4
C.D.	100,000 MLD.		(7)	7	(32)	16	(53)	89	(27)	46	(32)	16
C.J.M.		100,000 MLD.	(8)	8	(18)	9	(26)	44	(48)	81	(10)	10
T.L.C.	100,000 MLD.		(10)	10							(112)	57
W.G.D.		100,000 MLD.	(7)	7			(31)	52	(23)	39	(32)	16
V.G.		100,000 MLD.	(19)	19							(79)	40

Explanation: MLD. Minimal lethal dose for a mouse.

TABLE XII.
IMMUNIZATION OF MAN WITH A KNOWN QUANTITY OF CULTIVATED VIRUS AND A KNOWN AMOUNT OF IMMUNE SERUM.

Number of man.	Age of man.	Weight of man in kg.	Inoculum of virus.*		Inoculum of serum.*			Presence of circulating virus.	Febrile reaction.		Immunity.
			Quantity.	Subculture.	Amount in c.c. per kilogram.	Total amount in c.c.	Titre.		Days of fever.	Height of fever.	
1	39	89.5	110,000 MLD.	82, 83	0.6	53.7	88	—	3rd 4th	37.5° C. 37.5° C.	24†
2	30	64	170,000 MLD.	83	0.6	38.4	128	—	3rd 4th	37.2° C. 37.7° C.	36†
3	47	60	170,000 MLD.	83	0.6	36	128		2nd, 3rd, 4th, 5th	37.2, 37.3° C. 37.7, 37.4° C.	
4	49	68	170,000 MLD.	83	0.6	41	128		0		
5	33	63	170,000 MLD.	83	0.6	38	128		0		
6	32	63	170,000 MLD.	83	0.6	38	128		0		
7§		48.6	170,000 MLD.	83	0.5	24.3	256	—	0		+
8§		70.2	170,000 MLD.	83	0.5	36.1	256	—	4th	37.3° C.	+
9§		66.6	170,000 MLD.	83	0.5	33.3	256	—	0		+
10§		58.5	170,000 MLD.	83	0.5	29.3	256		0		+
11§		56	170,000 MLD.	83	0.5	28	256		3rd	37.7° C.	
12	37	86	40,000 MLD.	113	0.6	52	97		0		
13	36	55	40,000 MLD.	113	0.6	33	97		0		

14	45	71	40,000 MLD.	113	0.6	43	97		0		
15	10	43	40,000 MLD.	113	0.6	26	97		2nd 6th	37.2° C. 37.2° C.	
16	8	30	40,000 MLD.	113	0.6	18	97		3rd 6th	37.2° C. 37.2° C.	
17	39	70	40,000 MLD.	113	0.6	42	97		0		38
18	33	59	25,000 MLD.	115	0.6	36	97		0		
19	30	51	25,000 MLD.	115	0.6	31	97		0		
20	43	53	25,000 MLD.	115	0.6	32	97		0		
21	53	74	14,000 MLD.	120	0.6	45	97		0		256¶
22	44	66	14,000 MLD.	120	0.6	40	97		0		34**
23	20	48	14,000 MLD.	120	0.6	29	97		0		90**
24	22	54	14,000 MLD.	120	0.6	32	97		1	37.2° C.	38**
25	42	61	37,400 MLD.	124	0.6	37	94		0		+
26	34	66	37,400 MLD.	124	0.6	37	94		0		+

Explanations : *

Administered subcutaneously.

Minimal lethal dose for a mouse.

Reading at 28 days after inoculation.

Reading at 14 days after inoculation.

Inoculated in London by Dr. G. M. FINDLAY.

+ Present.

— Absent.

|| Reading at 21 days after inoculation.

¶ Reading at 18 days after inoculation.

** Reading at 21 days after inoculation.

The writers wish to acknowledge their indebtedness to Dr. W. A. SAWYER for much constructive criticism, and to Dr. A. F. MAHAFFY, H. L. FREESE, and W. G. CASSERES for considerable active help in the accomplishment of this work.

SUMMARY.

1. A pantropic or natural strain of yellow fever virus has been cultivated during a period of 21 months and through more than 150 subcultures *in vitro* without intercurrent animal passage.

2. A simple and practical technique for the cultivation of yellow fever virus is described.

3. Pantropic yellow fever virus has been cultivated for more than 130 subcultures in media consisting of serum-Tyrode solution and minced tissue of mouse embryo, chicken embryo or adult mouse testis. The same strain of virus has been grown for more than 55 passages in a medium of serum-Tyrode solution and minced adult guineapig testicular tissue, and for more than 20 transfers in the same fluid medium and chicken embryo dermis.

4. Neurotropic yellow fever virus of both relatively early and late mouse passage has been cultivated for more than 120 subcultures in a medium of serum-Tyrode solution and minced chicken embryo tissue. Neurotropic yellow fever virus of late mouse passage has been grown for 55 transfers in a medium of serum-Tyrode solution and minced mouse embryonic tissue. Neurotropic yellow fever virus of late mouse passage has been cultivated for 86 subcultures in a medium of Tyrode solution and minced chicken embryo tissue.

5. The cultivated strains of pantropic yellow fever virus have exhibited consistently in *Macacus rhesus* a progressive loss of the power to provoke yellow fever following inoculation. Evidence is advanced to show that the degree of neurotropism for mice and monkeys of pantropic strains of yellow fever virus does not increase during cultivation in various tissue media. Experimental data upon the virulence of a pantropic strain from the time of its isolation from man through its passage in monkeys, its cultivation in mouse embryonic tissue and its passage again through monkeys is presented.

6. A strain of pantropic yellow fever virus grown for 92 subcultures in a medium of serum-Tyrode solution and mouse embryonic tissue when passaged for 30 transfers in *Macacus rhesus* only slowly regained its virulence for monkeys. The increase of virulence thus provoked was manifested by the death of 6 of 30 monkeys in the series of yellow fever. None died of yellow fever virus encephalitis.

7. Monkeys become highly immunized against yellow fever if inoculated with the pantropic virus cultivated in mouse embryonic tissue together with human immune serum. Demonstrable serum immunity is produced even if the

inoculum of virus contains no more than 34 minimal lethal doses for a mouse and the amount of immune serum (titre 128) equals 5.0 c.e. per kilogram of body weight. The concomitant injection of human immune serum (titre 128) in a ratio of 0.5 c.e. per kilogram of body weight completely or nearly completely protects monkeys against the demonstration of circulating virus from the day of inoculation to the appearance of serum immunity.

8. The antigenic power for immune man of the neurotropic virus of mouse brain origin and the cultivated pantropic virus of mouse embryonic tissue origin is approximately the same. For previously immunized man the serum antibody titre rises rapidly following inoculation of virus, to reach a peak usually at 2 weeks, and falls rapidly thereafter to approach its initial level at 4 weeks.

9. The results of the immunization of 26 persons with pantropic yellow fever virus cultivated in mouse embryonic tissue, in the presence of an existing passive immunity produced by the concomitant injection of a titrated quantity of human immune serum are recorded. The reactions following inoculation in this short series were minimal or absent. The sera of thirteen individuals in this group which were titrated for protecting antibodies during the period from 14 to 28 days following inoculation showed titres ranging from 8 to +256.

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INFLUENCE OF CLIMATE ON SUSCEPTIBILITY TO ENTERIC INFECTIONS.

BY

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INTRODUCTION.

Hypothesis is the basis of all scientific progress. However, it is useful only to the extent that it serves as a means to an end ; if it becomes an end in itself, it may block rather than further scientific development.

In the study of disease and the factors concerned in its causation, two principal hypotheses have at one time or another alternately dominated medical thought and practice. One hypothesis was that disease was caused by extrinsic agents ; the other that it was induced by changes in the body humours.

The idea of an extrinsic cause assumes many forms. The earliest form is the curse of the gods, or the malign influence of the stars. The idea of contagion is first expressed in the Bible, and with it came the idea of quarantine and isolation. Later this idea was lost sight of and the doctrine of miasmas conveyed through the atmosphere became the dominant idea. It was not until the work of PASTEUR that the germ theory of disease became concrete, and as a working hypothesis led to discoveries which in a quarter of a century revolutionized medicine.

The second hypothesis which has dominated medical thought from classical times is that of the individual constitution. The constitution was not only supposed to determine susceptibility to disease, but also the character, symptoms and lesions. The Greeks based upon this theory the cult of physical hygiene. SYDENHAM built upon a modified form of this idea his theory of epidemiology based on the conception that with certain changes in the earth there were constitutional changes which rendered man susceptible to this or that disease.

In modern times these originally vague hypotheses—vague because of lack of knowledge—have assumed newer, clearly defined and more concrete forms. On the one hand, there is the well defined concept of infection and contagion and, on the other, there is recognition of the fact that different races, different individuals and, indeed, the same individual at different times, may be more or less susceptible to an infection with one or another organism. In the first glow of the rise of the idea of infection, it seemed that the answer to all epidemiological problems was at hand ; that epidemicity was determined solely by the virulence of the invading organisms and their number. Slowly it has become apparent that, for certain diseases at least, season, climate and individual predisposition

are important and that a complete understanding and solution of these epidemiological problems will only come when all the segments that make up the picture can be properly put together.

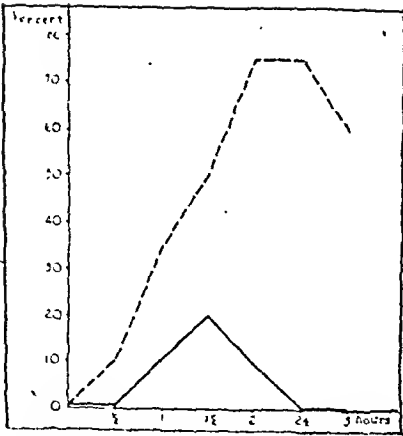
Accumulating observations have made it clear that individual predisposition is a variable factor. They have led to the working hypothesis that man (and animals) is not a stable organism, but a delicately balanced, physiological mechanism and that various disturbances in the physiological balance may bring about a change in his predisposition to infection. This working hypothesis has led to a study of the factors, internal and external, which by their effect on the body modify its susceptibility to infection. These studies cover a wide range—effect of environment, of diet, of internal secretions, etc.

The first two factors—climate and nutrition—are of particular importance in Palestine where a new population, coming from a temperate climate, is seeking acclimatization. And it is partly for this reason that we have devoted ourselves to these studies, some aspects of which I shall attempt to review briefly.

In studying the effect of climate, one has to take into consideration its various elements, such as temperature, humidity, air velocity and light, both singly and in combination. In nature it is not possible to separate these elements; all of them operate at the same time and the effect produced is the resultant of the various forces. In the laboratory it is possible to analyse the effect of the various factors individually and in combinations, but there is the drawback that it is not always possible to reproduce experimentally the combined conditions existing in nature. Moreover, the effect observed in experimental animals need not necessarily be identical with that which would be produced in man. Nevertheless, many interesting observations have been made in animal experiments which throw light on epidemiological events and on pathological changes noted in man. And it is the object of this paper to summarize the results of these experiments and to indicate their possible application to human adaptation to a subtropical climate.

EFFECT OF TEMPERATURE AND HUMIDITY.

SALLE (1911), showed that an increase in external temperature is followed by a rise in body temperature, by a decrease in total gastric secretion, in total and free acid and in pepsin. GRÜNFELDER (1914), showed that the latter was also true in fever. ARNOLD (1929), confirmed these findings and showed further that in animals kept in a warm, humid room there is a change in the enteric flora, the faecal flora of the large intestine ascending to the lumen of the duodenum. He found that with an alteration in the acid-base balance in the gastric tract towards the alkaline side, there was an appearance of a typical colon flora in the duodenum. His results indicate that the acid of the stomach has a bacteriostatic effect on the bacteria in the stomach and duodenum and that this effect disappeared with the reduced activity of the stomach induced by exposure to a hot, humid environment.



GRAPH 1.—Ordinate : percentage of *B. prodigiosus* appearing in caecum after intra-duodenal injection. Abscissa : time in half-hour intervals. Continuous line represents relative concentration of *B. prodigiosus* in ordinary room temperature. Broken line represents same in hot and humid room. Each graph is an average of nine experiments ; three experiments were repeated using three different dogs. (ARNOLD, 1929.)

In another series of experiments, ARNOLD and BRODY (1927), showed that if bacteria are injected directly into the duodenum of a dog kept in a warm, humid room they appear in the caecum, but not if the dog is kept in a cool room. Since in these experiments gastric acidity is excluded, one must assume that there is another factor besides gastric acidity ; or, in other words, that the humid heat causes a change in the bacteriostatic action of the intestinal mucosa. Graph 1 and Table I, reproduced from the paper by ARNOLD (1929) illustrate some of his findings.

In our experiments we approached the problem from another angle. ARNOLD studied the effect of a hot, humid atmosphere on stomach-intestinal bacteriostasis. Our own experiments dealt with the effect of various temperatures and humidities on the susceptibility of animals to infection. Comparable groups of mice were kept at different temperatures and humidities, infected *per os*

TABLE I.

DISTRIBUTION OF *B. prodigiosus* THROUGH GASTRO-INTESTINAL TRACT OF 3-MONTHS-OLD PUPPIES, $2\frac{1}{2}$ HOURS AFTER FEEDING WITH plain AND enteritidis MEAT IN COOL AND HOT ROOMS.

Food.	Stomach.	Duodenum.	Jejunum.		Ileum.	Caecum.
			Upper.	Lower.		
Control, plain meat ; cool room	0	0	0	0	0	10
Control, plain meat ; hot room	15	15	25	35	100	100
Enteritidis meat ; cool room	0	0	0	0	10	25
Enteritidis meat ; hot room	15	25	25	50	100	100
Heated enteritidis meat ; cool room	0	0	0	25	35	35
Heated enteritidis meat ; hot room	100	100	100	100	100	100

Figures indicate the percentage of dogs in which the organisms were recovered in the regions of the alimentary canal indicated. Cool room = room temperature ; hot room = temperature 95° to 98° F. ; relative humidity, 85 to 95 per cent.

with the same dose of *S. enteritidis*, and the course of infection noted. In addition to noting the mortality we also recorded the percentage and intensity of infection of the different organs of the animals dissected at various intervals after the infection. The results, summarized in Table II below, show that the fatality at a high temperature and high humidity (low cooling rate) is almost three times that at the same temperature associated with a low relative humidity (KLIGLER and OLITZKI, 1931).

TABLE II.

RESULTS OF INFECTION OF WHITE MICE KEPT AT DIFFERENT TEMPERATURES AND HUMIDITIES AND INFECTED WITH *S. enteritidis*.

Days after infection...	14 to 30
Temperature—C.	13°	20°		30°	
Relative humidity—per cent.	90	35	95	35	95
Number of animals	56	39	56	56	39
Total infected*	36	23	31	27	18
Total dead	15	9	14	6	12
Total sterile*	5	7	11	23	9
Total liver abscesses	10	1	3	1	1
Fatality—per cent.	25	23	25	10·7	30·7
Sterile—per cent.	9	18	19·6	41	23

* At time of dissection.

These findings were subsequently confirmed by ROBERTSON and WELD (1932), who infected rachitic rats with *S. enteritidis*. In three different experiments they found that at a high temperature and low humidity, 55, 56, and 74 per cent. of the rats survived, while in the corresponding groups kept at the same temperature associated with a high relative humidity, 0, 25, and 52 per cent. survived.

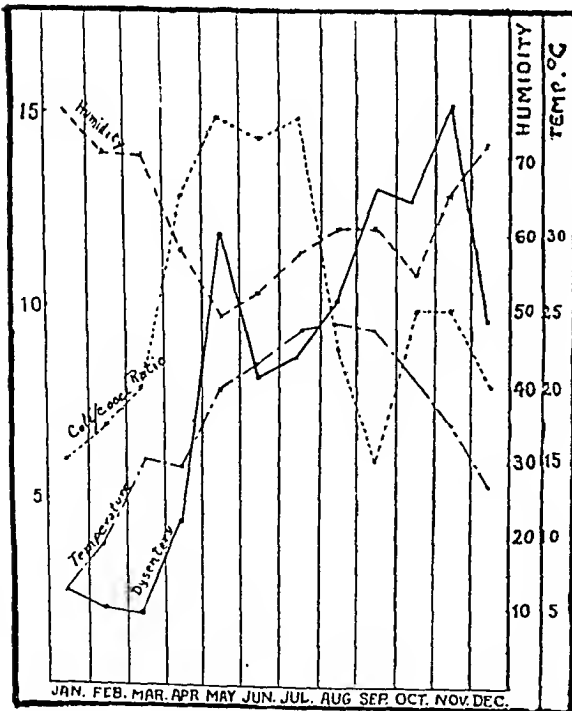
It is apparent then that a high temperature associated with a high humidity, or in other words a reduced cooling power, is particularly harmful and that under these conditions the resistance of both normal and rachitic animals is so reduced as to raise the mortality two to threefold.

The various experiments, a number of which are cited above, show that a high temperature associated with a high humidity, favours the passage of infecting organisms across the stomach-duodenal barrier, increases the permeability of the intestine so that the bacteria pass into the lymphatics and finally reduces the defence of the animal to such an extent that the severity of the infection is enhanced.

The relation of these experimental findings to the epidemiology of intestinal infections is of interest. It is known that enteric infections, dysenterics, diarrhoeas and colitis increase during the spring and summer months and diminish during the winter. This increase, which is often sharp and sudden, is ascribable to the sudden rise in the external temperature which presumably causes a retardation in the gastric secretions and allows either infectious organisms or irritating toxic substances to pass more readily into the lower intestinal tract. By reducing the bacteriostatic action and increasing the permeability of the intestinal mucosa, infectious organisms can either more readily become localized on the intestinal mucosa, or penetrate the wall and cause infection. Furthermore, it would appear that at a high humid temperature the intestinal tract is also more sensitive to the irritating substances produced by the normal intestinal flora. MILLS (1928), in China, has called attention to the gastro-intestinal disturbances, associated with nausea and vomiting, following a sudden elevation in temperature and humidity; this occurred particularly among foreigners. Similar effects are often observed in Egypt. A sudden change to high, humid temperature may, therefore, facilitate infection, or give rise to non-specific irritation.

We also approached this problem from another angle and studied the character of the intestinal flora of normal individuals in different months of the year. The study was limited to a dozen people, but it covered a period of 20 months and involved the examination of 117 stools. Some of the data are reproduced in Table III, p. 536 (GOLDWASSER and KLIGLER, 1930).

Allowing for variations due to technique this study shows a number of interesting trends which are in harmony with the experimental findings already referred to. Three sets of data seem particularly significant, because they indicate a change in the intestinal tract which is correlated with the seasonal changes in temperature and humidity. It will be noted from the table that the faecal moisture begins to rise in April and drops again during November to March. At the same time, particularly during the months of April to July, there is a reversal in ratio of *B. coli* to *Streptococcus*



GRAPH 2.—Correlation of the colon-cocci ratio and dysentery curves and their relation to temperature and relative humidity.

and, inversely, with that there is a drop in the anaerobic flora. In other words, beginning in April, a change takes place in the intestinal tract which is favourable to the development of a *B. coli* flora. The significant point is that at the same time there is a sharp rise in the daily temperature and the annual beginning of the epidemic rise of dysenteric infections and diarrhoeas. This relationship is brought out in Graph 2 (p. 535).

TABLE III.
MONTHLY VARIATIONS IN THE FAECAL FLORA OF NORMAL INDIVIDUALS.

Month.	Bacteria per gramme Dry Faeces : in Millions.	Percentage of Moisture.	Ratio of <i>B. coli</i> to Cocci.	Anaerobes Present (Dilution 1 : 10,000).
January	73,000	50	0.6	+
February	87,000	49	0.8	+
March	91,000	49	0.9	+
April	65,000	58	1.1	±
May	29,000	60	1.5	±
June	48,000	63	1.7	—
July	50,000	61	1.8	±
September	66,000	60	0.6	±
October	55,000	63	1.0	±
November	70,000	51	1.0	+
December	87,000	51	0.8	+
January	156,000	47	0.6	+
February	159,000	47	0.6	+
March	142,000	46	0.7	±
April	109,000	56	1.5	±
May	75,000	56	1.5	±
June	91,000	63	1.2	±
July	96,000	60	1.2	±
August	143,000	59	0.9	±

These independent data, obtained by approaching the problem from different angles, converge to one point, namely : that associated with the sudden rise in temperature there are changes in the intestinal tract which favour the development of saprophytic and pathogenic members of the colon-typhoid group. These results suggest further that it is rather the sharp deviation in temperature which causes the disturbances, because during the height of the summer months—August to September—the organism again tends to reach an equilibrium.

The cumulative results indicate, therefore, a seasonal flux in the condition of the intestinal tract of man, while the experimental data obtained with animals show clearly that corresponding changes are induced in them by a change from an environment with a high to one of low cooling power.

The explanation of this effect of external climate on the intestinal tract is probably to be sought in the disturbances of the equilibrium, known to exist, between the vessels of the peripheral areas of the body and those of the splanchnic region. When there is a vaso-dilatation of the skin capillaries there is vascular constriction in the splanchnic area and *vice versa*. An increased blood supply in the splanchnic region is associated with an increase in the gastric and bile secretions (MÜLLER and PETERSEN, 1926 ; PETERSEN and MÜLLER, 1927) and an increase in concentration of the leucocytes in the blood in that region (MUELLER, 1926). During this time there is vaso-constriction and leucopenia in the peripheral vessels. On the other hand, when there is vaso-dilatation in the periphery, there is a leucocytosis and an increased activity in the skin glands ; while at the same time there is leucopenia and diminished activity in the splanchnic region.

Since high temperature and humidity or low cooling power cause a dilatation of the peripheral vessels, there is at the same time a reduced blood supply in the splanchnic region ; consequently, there is a reduction in gastric activity as well as in the activity of the internal organs (bile secretion, glands), and their bactericidal powers are diminished.

HIGH DRY TEMPERATURE.

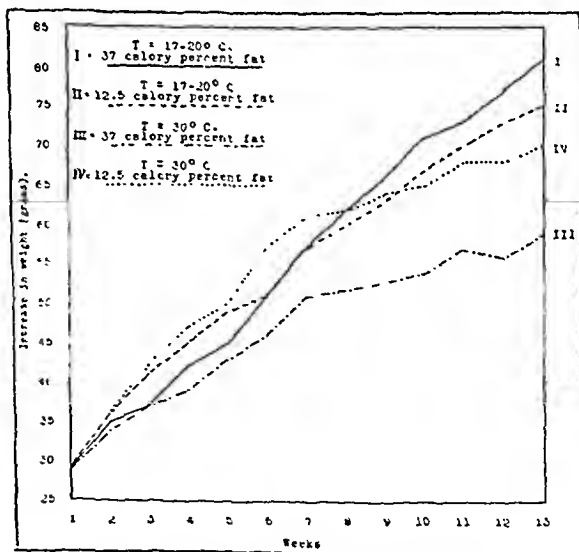
Another point to be considered in this connection is the effect of high, dry, temperatures. This problem is particularly important because while we know the baneful effects of "hamsins" or siroccos, the mechanism of their action is not clearly understood. It is conceivable that at humid, warm, temperatures the intestinal tract is rendered vulnerable as a result of an impoverished blood supply to the splanchnic region. But experiments in progress as well as our epidemiological observations indicate that the bacteriostatic action of the intestine is also reduced by the excessively dry heat characterizing our "hamsins." This effect is, apparently, due to an excessive loss of water leading to a marked anhydremia. This is particularly significant in view of the recent findings by COWGILL and his associates (1930) that anhydremia caused a reduction in

gastric mobility. According to these authors, stomach function can be affected by excessive loss of water irrespective of temperature. In our case, however, we are concerned with an anhydremia due to water-loss caused by a high temperature associated with an excessively low humidity. The two climatic extremes for different reasons produce the same effect.

TEMPERATURE AND NUTRITION.

There is also a third aspect of the problem. Temperature exerts an indirect influence on the body by affecting its state of nutrition. In our experiments on the relative value of animal and vegetable fats we noted (KLIGLER, GEIGER and MUELLER, 1932) that young rats receiving 25 to 35 per cent. of their calorie requirement in the form of fat showed retarded growth and, after a few weeks, developed a cachectic appearance. This effect was most marked during the summer months. It seemed, therefore, that at a high environmental temperature, a high fat content was injurious to the organism. Pursuing these studies under controlled conditions, it developed that if parallel groups of animals were kept on the same diet having a high fat content, one at a low, the other at a high, temperature, there was a decided difference in their respective growth and development. Those kept in a warm temperature fell below those in a cool temperature. It was found further that a relative increase in the protein improved the growth to some extent, but that the harmful effect noted at high temperatures could only be counteracted by an increase of vitamin B in the diet (Graphs 3 and 4).

The explanation of these observations was not apparent. At first we thought that at high temperatures there was a disturbance in the fat utilization resulting in an acidosis, and, furthermore, that the animal required more vitamin B at high than at low temperatures. Experiments now in progress have shown, however, that the explanation is much more simple. In these experiments the vitamin B is mixed with the food, the animals thus receiving an amount of vitamin proportional to the food intake. Animals kept at a high temperature and fed *ad libitum*

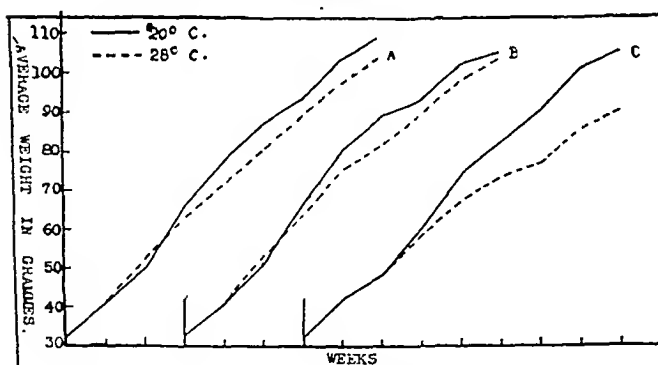


GRAPH 3.—Influence of high fat diets on growth of rats kept at various temperatures.

consume, however, less food than those kept at low temperatures. Depending on the diets used, the difference in the amounts consumed may range between 20 and 30 per cent. When the experiments were modified so that the animals kept at the high temperatures received a supplement of vitamin B, so that the total amount was the same as that received by those kept at low temperatures, corresponding groups developed equally well. Not only that but, when the total amount of vitamin B was kept below the optimum level, the low temperature animals which consumed more food, that is had a higher metabolic rate, developed avitaminosis one or two weeks earlier than did the warm temperature animals. The following experiment is illustrative:—

Equal groups of rats were placed at room temperature (20 to 22° C.), and incubator temperature (28° C.), and given the following diets:—

	A (High Protein) Low Fat. Grammes.	B (High Protein) High Fat. Grammes.	C (Low Protein) High Fat. Grammes.
Starch	540	416	456
Casein	160 (16 per cent. of total calories)	160 (16 per cent. of total calories)	120 (12 per cent. of total calories)
Sugar	120	80	80
Olive oil	55 (12 per cent. of total calories)	146 (33 per cent. of total calories)	146 (33 per cent. of total calories)
Salt mixture (Osborne and Mendel)	40	40	40



GRAPH 4.—Growth curves of rats kept on the same diets at high and low temperatures respectively, and receiving an amount of vitamin B adequate for optimum growth under ordinary conditions.

- A = High protein (16 calories per cent.).
 Low fat (12 " ").
 B = High protein (16 " ").
 High fat (33 " ").
 C = Low protein (12 " ").
 High fat (33 " ").

Each diet was subdivided into two parts; to one 100 grammes of marmite was added and to the other only 50 grammes. Other vitamin supplements (cod liver oil and lemon juice) were the same. The growth of the rats receiving the diet with 100 c.c. of marmite is shown in the attached curve (Graph 4).

It will be noted that when the diet contained a moderate amount of protein (16 calories per cent.) and an adequate amount of vitamin B, the growth at the two temperatures was practically the same.

When, however, the amount of protein was low and fat high, even this amount of vitamin did not compensate and the growth of the rats at the high temperature fell considerably below that of the cold temperature group. When the vitamin content was inadequate, the growth of the high temperature rats fell, in all cases, below that of the corresponding cold temperature group.

A comparison of the amount of food consumed daily by each of the various groups during the last week of the experiment is instructive. The figures were as follows :—

TABLE IV.

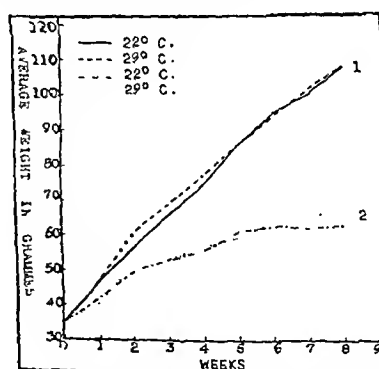
AMOUNT OF FOOD CONSUMED (IN GRAMMES).

	Five Rats receiving Diet with 100 grammes Marmite.			Five Rats receiving Diet with only 50 grammes Marmite.		
	A	B	C	A1	B1	C1
Incubator (28° to 30° C.)	40	35	40	17.6	20	18
Animal house (20° to 22° C.)	51	47	50.6	27.6	31	26.6
Percentage difference	21	25	21	36	35.5	40

It will be noted that when adequate vitamin B is given, the warm temperature rats consumed 21 to 25 per cent. less food ; but when the vitamin content was inadequate, the difference was 35 to 40 per cent. Even under optimum conditions the high temperature rats received 20 to 25 per cent. less vitamin B.

If now we take for comparison another experiment in which the 20 per cent. difference in the vitamin B intake was made good we obtain an entirely different picture : in this particular experiment only one diet—the C mixture was used. All rats were fed alike except that each day the incubator group received as supplement the amount of vitamin contained in the difference between the quantity of food consumed by the cold and warm groups respectively.

In this experiment the cold temperature animals receiving only half the amount of marmite were below the weight of those at the high temperature and loss of weight and paresis began to appear, when the warm temperature animals were still maintaining their weight (Graph 5).



GRAPH 5.—Growth curves of rats kept at high and low temperatures respectively, when percentage of vitamin B equal to difference in food intake is supplemented to warm animal group.
1 = adequate vitamin B ;
2 = inadequate vitamin B.

The difference in food intake is also of interest. It is given below.

	Five Rats receiving Diet with 100 grammes Marmite.		Five Rats receiving Diet with 50 grammes Marmite.	
	Grammes of Food per Five Rats Daily.		Grammes of Food per Five Rats Daily.	
	Beginning of Experiment.	End of Experiment.	Beginning of Experiment.	End of Experiment.
Incubator	27	28.5	16.5	13.5
Animal house	34	38.5	21	14
Percentage difference	20.6	26	21	0

The adequate vitamin group shows about the same difference in the amount of food consumed as in the previous experiment, whereas in the low vitamin group the difference dwindles, the low temperature group loses appetite and its weight falls below that of the high temperature group.

It seems clear, therefore, what transpires. At warm temperatures the animal organism requires less food than at cold temperatures; the basal metabolism is lower (SUNDSTROEM, 1927, 1930). Since the animals kept in a warm temperature consume 20 to 25 per cent. less food than their companions kept at low temperatures on the same diet, they receive also with the lower food intake about 20 to 25 per cent. less vitamin B. This difference is particularly harmful when the fat content is high and protein content low. Since the amount of vitamin B required is, as shown by COWGILL (1932), proportional both to body weight and metabolic rate, a reduced intake of vitamin B below the required amount results in a loss of appetite with a further reduction both in the food and vitamin B intake. (See Table IV, p. 540.) A vicious circle is thus established and progressive avitaminosis and inanition result.

When there is an excess of vitamin B in the diet, this effect is not noted. However, when the amount of vitamin B is just above the requirement threshold for low temperatures, there is a retardation in growth in the high temperature group due to reduced food intake and loss of appetite and in the course of time avitaminosis is sure to set in.

The investigations conducted by the New York State Ventilation Commission have shown that in human subjects as well there is a lowered basal metabolism at high temperatures. Summarizing these findings WINSLOW (1927), the Chairman of the Commission, writes: "One of the most important contributions of these investigations is the recent report that the basal metabolism decreases with increased temperature up to about 26° C. (with 100 per cent. relative humidity) and then increases, the rise becoming very rapid when the atmospheric temperature is higher than that of the body." Since 26° C. and

100 per cent. relative humidity is equivalent to about 31° C. and 50 per cent. relative humidity, our results with rats correspond closely with those observed in humans.

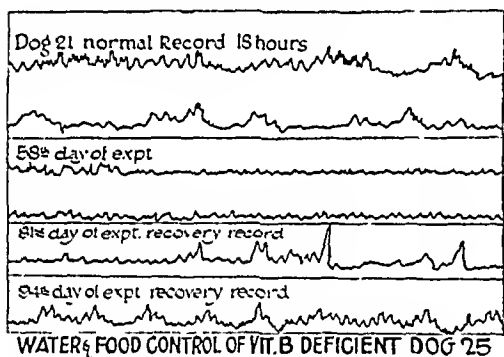
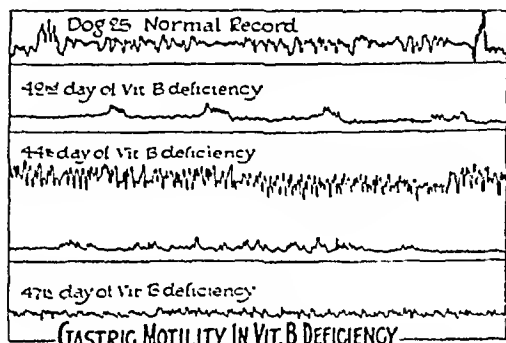
Of especial interest is the fact that at the same time as the symptoms of avitaminosis develop, the animals become more susceptible to an enteric infection. This is illustrated by the following experiment. Two groups of rats were kept at 20° and 28° C. respectively, and fed with different amounts of vitamin B. The rats at the high temperature were provided a favourable humidity (40 to 45 per cent.). At the end of eight weeks, when symptoms of avitaminosis (loss of weight and early signs of paresis) developed, the animals were infected *per os* with a strain of Gaertner bacillus. All those which died and had not been devoured were cultured. After two weeks all animals were killed and cultures made of the heart, liver, spleen and mesenteric glands.

The results are given in Table V. They show a striking difference in the susceptibility of rats kept on a vitamin B-poor and vitamin B-rich diet. These experiments are still in progress, but the data are suggestive. At the low temperature twelve of sixteen of the vitamin B-rich rats were negative against four out of fourteen of the vitamin-poor animals. At the high temperature eleven out of fifteen were negative as against five out of thirteen. Irrespective, therefore, of the temperature, B avitaminosis seems to lower the resistance of the animals to an enteric infection.

TABLE V.

Diet.	Animal House (20° to 22° C.).		Incubator (28° C.).	
	Vitamin Rich.	Vitamin Poor.	Vitamin Rich.	Vitamin Poor.
High protein Low fat	3 negative 1 mesentery 1 sepsis	2 devoured 1 sepsis 1 organs	5 negative	2 negative 1 devoured 1 mesentery
High protein High fat	4 negative 1 mesentery (1 colony)	4 negative 1 sepsis	2 negative 1 sepsis 2 mesentery	1 devoured 2 spleen 1 mesentery
Low protein High fat	5 negative	3 sepsis 2 devoured	4 negative 1 mesentery	3 negative 1 sepsis 1 spleen
Total	12 negative 1 sepsis 2 mesentery	4 negative 5 sepsis 1 organs positive 4 devoured	1 sepsis 3 mesentery 11 negative	5 negative 2 devoured 1 sepsis 3 organs positive 2 mesentery
McCollum Standard diet	10 negative		8 negative 1 organs positive	

The explanation of these results is again to be found in the work of COWGILL (1930) and his associates. These investigators have shown that one of the consequences of B avitaminosis is a reduced gastric motility (see Graph 6) and, furthermore, that the loss of gastric motility was probably due to the anhydremia which is associated with the avitaminotic state. The increased susceptibility to an enteric infection may, consequently, be attributed to the reduced gastric function.



GRAPH 6.—In these graphs are presented portions of the gastric tracings obtained with vitamin B-deficient Dog 25 and its water-and-food control companion, Dog 21 (ROSE, STUCKY and COWGILL, 1930).

associates in the treatment of toxicosis in infants. In some cases they have obtained striking results by the injection of vitamin B (1933), while more recently excellent results have been reported (GRUENFELDER, 1935) by the injection of large quantities of saline solution.

Evidently, the summer toxæmia in infants is due to an anhydremia caused either by malnutrition—a lack of vitamin B, or by the sudden dehydration due to the high, dry temperature or, what is more likely, by a combination of the two factors. The relation of the “hamsins” to toxicosis is recognized by all workers here. Eliminating the anhydremia removes the immediate cause of the toxæmia and leads to recovery.

We arrive then at the same result by two different routes: (1) A high temperature associated with an excessively low humidity leads to loss of water, to anhydremia, to reduced gastric onus, to increased susceptibility to gastric irritation and infection. (2) A high temperature, with moderate humidity, leads to reduced food intake, which may also result in a reduced vitamin B intake, to B avitaminosis, to anhydremia, to reduced gastric tonus and to an increased susceptibility to an enteric infection. In the first instance the change is sudden, while in the second, the anhydremia may develop only after a time. The end result is the same. It may well be that some of the gastric disturbances in infants as well as adults are due to a B avitaminosis, while others, particularly in infants, are ascribable to a sudden upset in the water balance caused by a high dry temperature.

This view is supported by the results obtained by GRUENFELDER and his

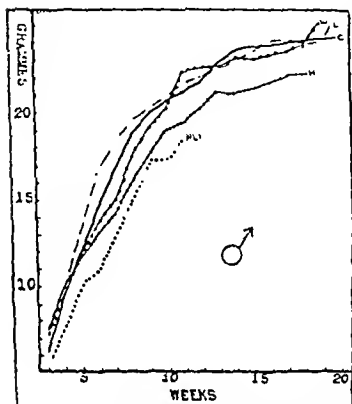
What practical inferences for tropical hygiene can be drawn from these experiments? Three points stand out clearly.

(1) In a hot humid climate it is of primary importance to cool the body and help it to maintain its normal temperature. Cooling the body surface (fans, showers), and stimulating the splanchnic region, particularly before a meal, would help maintain the proper balance between the peripheral and splanchnic circulation and assure normal gastric function.

(2) It is most important to insure a constant, adequate replenishment of water both to cool the body and, particularly, to counteract the tendency to anhydremia.

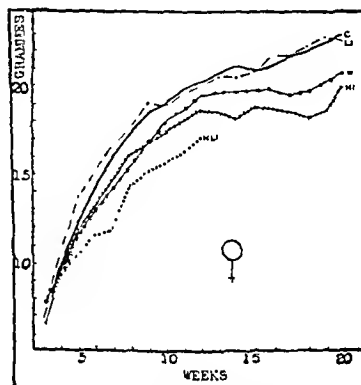
(3) In general in hot climates it should be remembered that vitamin B

requirement is constant and proportionate to the body weight as well as metabolism. And since the total food intake is less at high than at low temperatures, it is necessary to reduce the fat content in the diet and increase the protein and vitamin B containing foods. But whatever the diet, one should insure, during the summer and in hot climates generally, an adequate supplement of vitamin B. It is quite possible that loss of appetite in the summer is due, partly at least, to an inadequate intake of vitamin B.



GRAPH 7.—Comparison of growth curves of male mice under different climatic conditions (SUNDSTROEM, 1922).

C = Control group, cold; LI = Cold plus light; HI = Stagnant heat, subdued light; W = Stagnant heat plus wind (increased cooling effect); HLI = Stagnant heat plus light.



GRAPH 8.—Comparison of growth curves of female mice under different climatic conditions (SUNDSTROEM, 1922).

C = Control group; LI = Cold plus light; HI = Stagnant heat, subdued light; W = Stagnant heat plus wind (increased cooling effect); HLI = Stagnant heat plus light.

Light.—One other climatic element remains to be considered and that is light. We have attempted to study the effect of solar irradiation on the susceptibility of mice and rats to infection. We have also studied the literature on the subject. All one can say at present is that there are not as yet sufficient data to warrant conclusions, regarding the effect of light on the animal organism. Experiments on the effect of short periods of daily solar irradiation, conducted during the last three years, have indicated that the problem is a highly complex one and that one cannot treat of radiation alone without taking other climatic factors, such as temperature and humidity, into consideration (KLIGLER and OLITZKI, 1935; KLIGLER and COMAROFF, 1935).

In our experiments, short periods of daily solar irradiation were neither

harmful nor beneficial if care was taken to prevent overheating. However, at the high temperature prevailing during the summer, overheating is the rule and even short periods of irradiation decreased the resistance of the animals to an intravenous injection. It may, therefore, be surmised that excessive exposure to direct solar irradiation under our conditions is harmful, if for no other reason than that the resulting overheating increases the load on an already overloaded organism trying hard to regulate its temperature and maintain a normal equilibrium.

In this connection it is of interest to refer to the experiments conducted by SUNDSTROEM (1922), on the effect of exposure of mice to artificial light in a cold and a hot-humid environment respectively. He found that at the high humid temperature, light adds to the retarding effect of such an environment on growth, and that this unfavourable effect may partly be neutralized by circulating the hot air, that is by increasing the cooling power. However, at ordinary room temperature, light had a slight stimulating effect on growth. The charts reproduced here summarise some of his experiments (Graphs 7 and 8, p. 544).

It is apparent that in a study of this problem one is beset with many difficulties and that a particular environmental factor may, under certain conditions, have a favourable effect, while under others, it may prove distinctly harmful.

SUMMARY.

Summarizing the data presented above, it appears that a warm tropical or subtropical environment may enhance susceptibility to an intestinal infection in three ways.

(1) The reduced cooling power of a hot humid climate causes a retardation in gastric secretion, breaking down the barrier to infection. It reduces the bacteriostatic action of the intestinal mucosa and increases the permeability of the intestinal wall to the infectious organism. It also increases the sensitivity of the intestines to toxic irritants.

(2) An excessively dry, hot climate may produce the same effect by causing a loss of water. The anhydremia thus produced again reduces stomach tonus and gastric activity and increases susceptibility to an enteric infection.

(3) A high temperature may produce the same effect indirectly by its influence on nutrition. Due to the lower food requirement at high temperatures, there may also be an inadequate intake of vitamin B. This in turn leads to a loss of appetite and may ultimately result in a state of B avitaminosis. This state leads to anhydremia, a reduced gastric tonus and an increased susceptibility to enteric infection.

(4) Under these conditions direct solar irradiation accentuates the effect of the hot environment. Consequently, exposure to direct sunlight, which may have a favourable effect on the organism in temperate climates, may produce the opposite effect in a hot-humid climate.

The experimental approach to these problems is only at the beginning, but sufficient data are at hand to indicate the importance of such research in the

elucidation of the effect of climate on the organism, human and animal, and on its resistance to infection.

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CELLULAR REACTION TO *BACILLUS LEPRÆ**.

BY

ERNEST MUIR, M.D.

The cellular reaction is of importance in all diseases caused by infective organisms. It is of peculiar interest in leprosy because of the low toxicity of the causal organism, *Bacillus leprae*. Due to this low toxicity the organisms can multiply to extraordinary numbers without causing very marked clinical signs, and without apparently interfering to any great extent with the general health of the patient.

If we compare leprosy with the sister disease, tuberculosis, the contrast is very striking. In the latter disease even a small infection sensitizes the patient so that an inoculation of tuberculin causes an immediate focal and general reaction. In leprosy the inoculation of leprosy bacilli causes no immediate focal and general reaction. Indeed the condition in leprosy may be compared with that in an advanced case of tuberculosis, in which the tuberculin reaction is negative; only, while such a tubercular patient is in a highly toxic condition and appears extremely ill, the leprosy patient shows few or no signs of toxicity, can go about his work in good health, and may even show but few clinical signs.

The disease of leprosy is not caused then by the toxins of the causal organisms, nor, except in the complication known as "lepra reaction" or lepra fever, is it due to sensitisation to the bacillus. The clinical signs and symptoms are caused by local cellular reaction to the bacillus, and it is the nature of this cellular reaction which I shall attempt to study in this paper.

Leprosy may affect most of the organs of the body, but it is the skin and the peripheral nerves that are most markedly involved, and we shall confine our attention to these structures.

Under certain circumstances such as in lepra fever, and when a severe temporary reaction takes place, the blood cells invade leprosy lesions and phagocytose the bacilli. But under ordinary conditions the cell responding to the presence of *B. leprae*, in both skin and nerves, is the endothelial cell of the capillary. It is difficult to say whether all endothelial cells respond, or whether, as some writers state, only certain specialised endothelial cells belonging to the reticulo-endothelial system are involved.

The multiplication of lepra bacilli which have entered the skin or nerves takes place in the intercellular lymph spaces and inside the cells. The degree of this multiplication, and the subsequent spread of the organisms throughout the tissues, are largely dependent on the degree of cellular response.

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The reaction of the cells to the bacilli in their neighbourhood is three-fold, *viz.*, increase of cells by division, ingestion of bacilli, and destruction of ingested bacilli. The more the bacilli multiply, and, therefore, the more bacilli there are in the neighbourhood of a cell, the more is that cell stimulated to respond. But apart from the number of bacilli surrounding it, the degree of response varies greatly in degree, and this variation is dependent on certain factors to be mentioned later.

Comparison of weak and strong response.—If the response is *weak* then cell-division is sluggish, and ingestion feeble, so that numbers of bacilli remain outside the cells and spread in the lymph stream; while the ingested bacilli, instead of being destroyed, multiply in the cytoplasm. These cells gradually become distended with bacilli, vacuolated and destroyed. The distended, vacuolated cell is the well-known "foamy" or "lepra" cell. In the nodules and other gross lesions groups of these cells are pressed together and may form a more or less uniform bacillary mass, cemented together by mucoid material called by the early leprologists "gloea." Between the typical lepra cells is a varying number of small round, lymphocyte-like cells, called by UNNA "daughter cells," suggesting that they constitute the young form of lepra cells.

If the cell-response to the bacilli is *strong*, then both cell-division and bacillary ingestion are active (Fig. 1). The cells are formed into compact cords round the capillaries (see Fig. 2), from the endothelial cells of which they take origin. These are the well-known "epithelioid cells" (see Figs. 3 and 4) of the so-called "tuberculoid" type of leprosy. The bacilli, instead of multiplying and distending the cells, are phagocytosed and destroyed; they are, therefore, few in number and difficult to find in such lesions. So active is the response in some cases that the cells, instead of dividing in the usual manner, form multi-nucleated giant cells, similar to the Langhan's cells in tuberculosis. This may be carried one step further and, as in tuberculosis lesions, result in caseation, which, when extensive, may lead to abscesses in the nerves and ulceration of the skin.

We have described shortly the contrasting pathological results in weak and strong cell response to *B. leprae*. Between these two extremes we have all grades of cellular activity. Moreover the degree of reacting power may vary in any case from time to time. Hence the protean forms of leprous lesions.

The principal factors influencing the degree of cellular reaction are four in number, *viz.*, (1) the age factor, (2) general health, (3) small infections with *B. leprae*, and (4) gross infection with *B. leprae*.

(1) *Age factor.*—It has long been recognised that young children are more susceptible to leprosy than adults. It is only recently that this important fact has been given due prominence, and its cause studied. The incidence among children in infectious contact is given as over 40 per cent., and conjugal infections as less than 5 per cent. It is generally recognised that a similar susceptibility of children also exists in tuberculosis. In the latter disease, the



FIG. 1.

FIG. 1.—Macule of back in patient with high resistance (N_2). The white mark denotes material removed by biopsy, a section of which is shown in Fig. 2. The resistance of this patient had been temporarily lowered, but had been restored shortly before the biopsy was made.

FIG. 2.—Section from macule in Fig. 1 showing skin and subcutaneous tissue. Note the sections of dense granulomatous cords round the vessels of the papillae, subpapillary plexus and hair follicle, which show white in the photograph; also the two thickened and granulomatous nerve branches in the subcutaneous tissue. The squares "a" and "b" in the latter indicate the areas shown enlarged in Figs. 3 and 4. No bacilli were found in the skin, but a few bacilli were found in the subcutaneous nerve at "a".

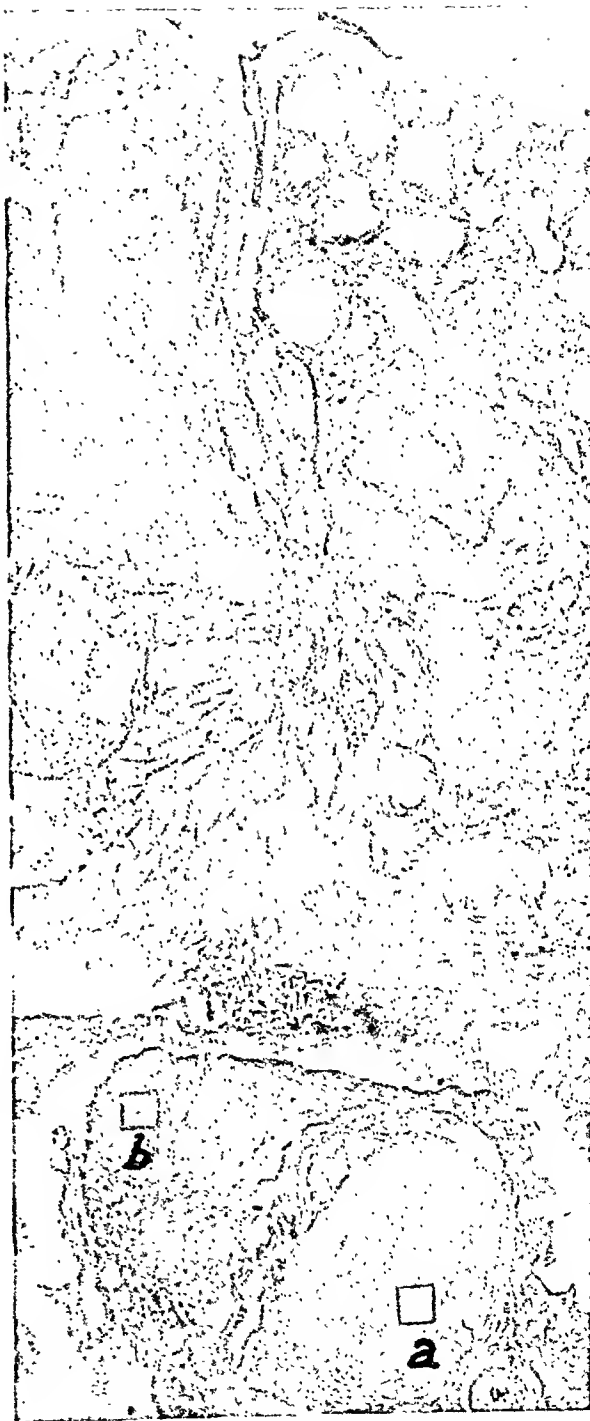


FIG. 2.

FIG. 3.

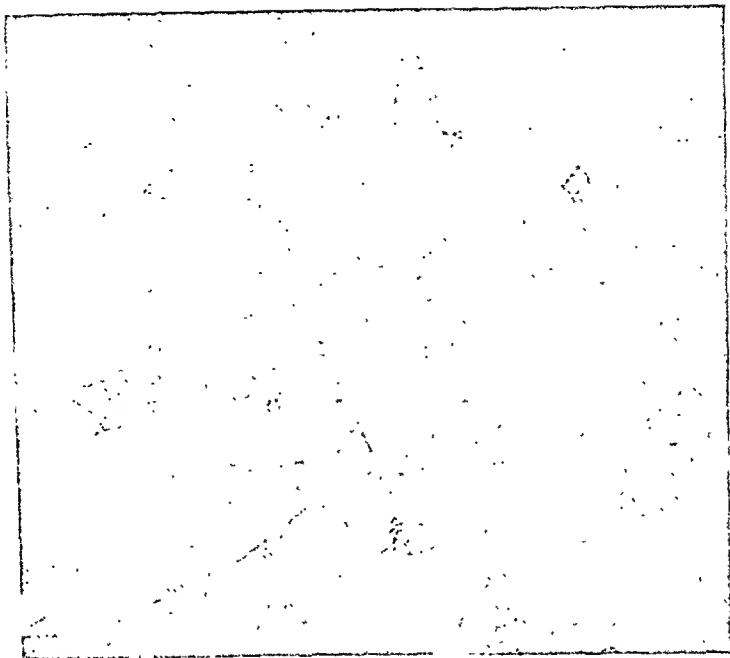


FIG. 4.

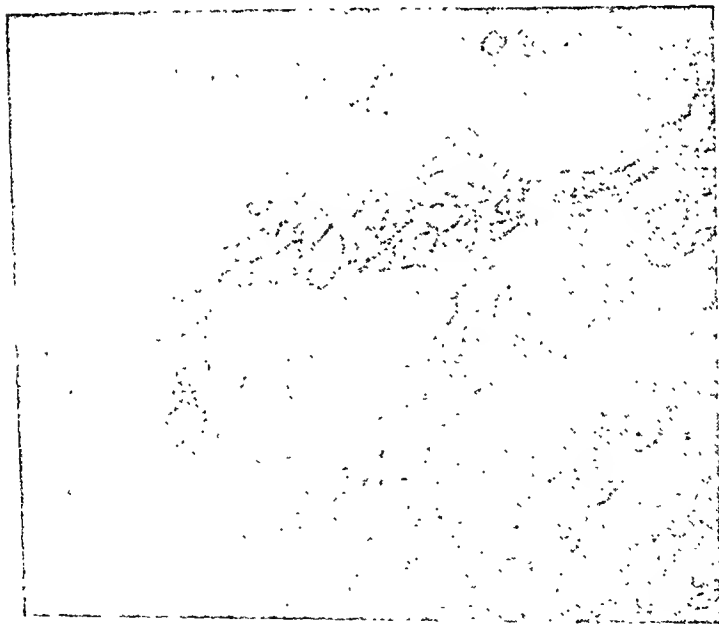


FIG. 3.—Section of subcutaneous nerve enlarged from "a" in Fig. 2. Note the few bacilli showing singly and in bunches ; and the large, densely packed epithelioid cells.

FIG. 4.—Section of subcutaneous nerve enlarged from "b" in Fig. 2. No bacilli were found in this nerve. Note the giant cell and the dense cellular reaction.

FIG. 5.

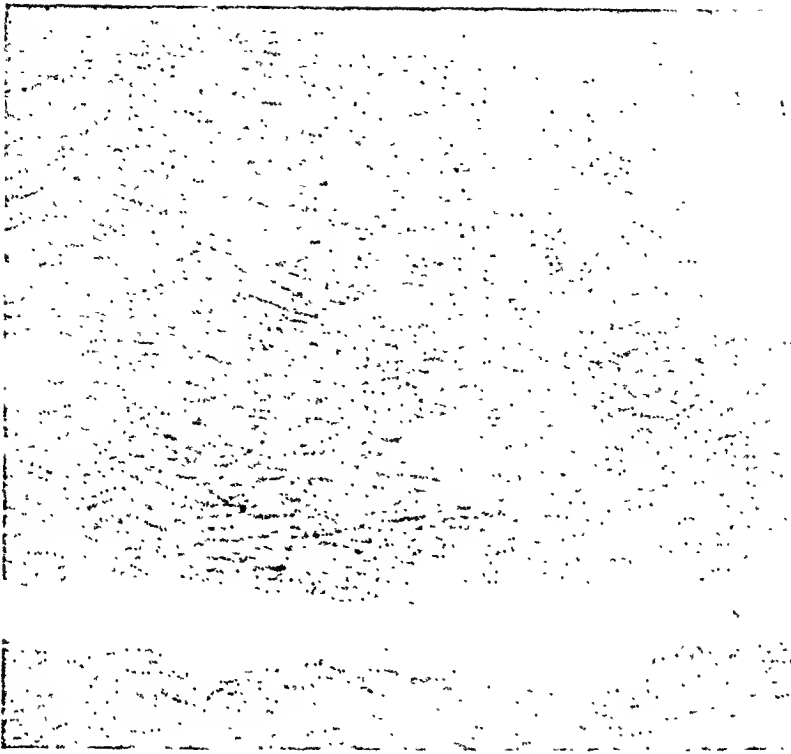


FIG. 6

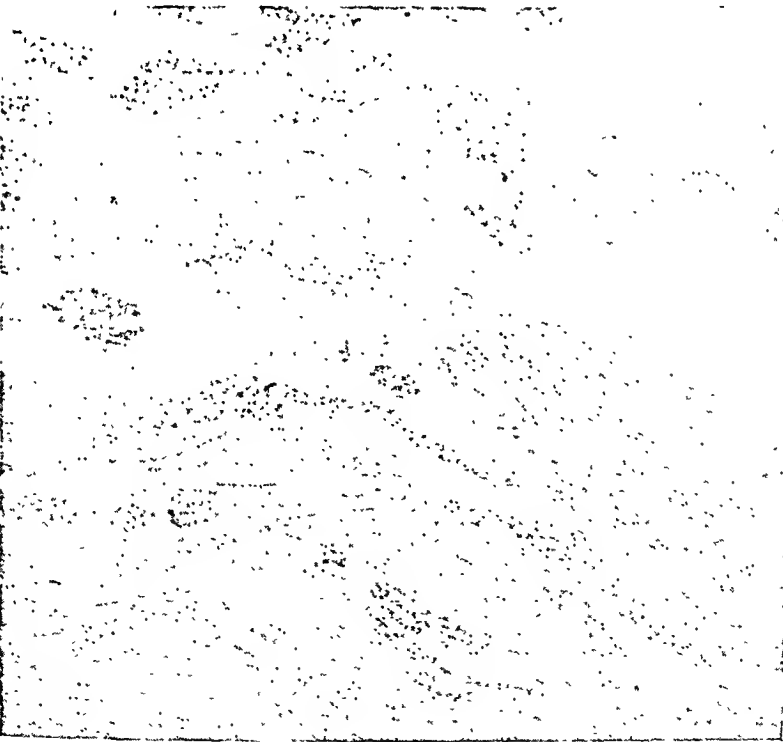


FIG. 5.—Section of nerve—low power—in case with low resistance (C_2). The section was stained by a combined Beilchowsky and Ziehl-Neelsen method. The dark dots are masses of bacilli lying between the nerve fibres. Cellular reaction is absent.

FIG. 6.—The nerve shown in Fig. 5, further enlarged. Note the masses of bacilli between the nerve fibres and the absence of cellular reaction.

FIG. 3.

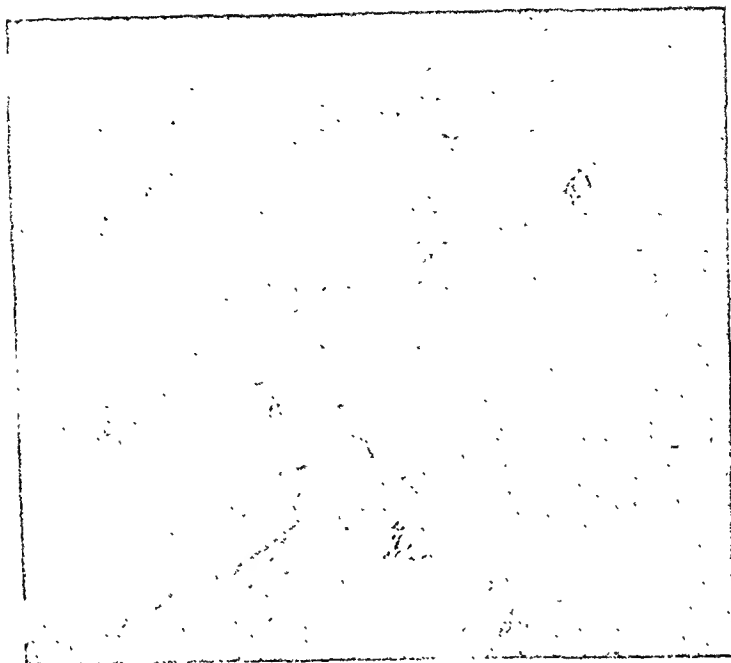


FIG. 4.

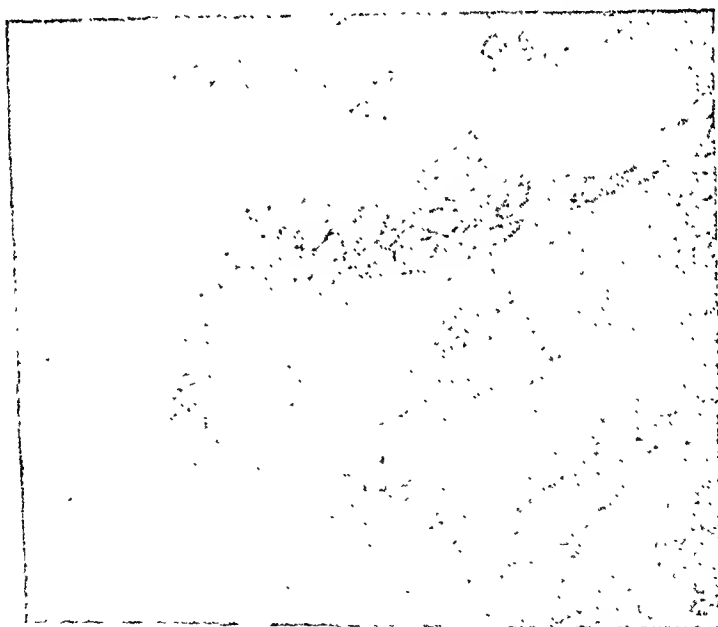


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FIG. 4.—Section of subcutaneous nerve enlarged from "b" in Fig. 2. No bacilli were found in this nerve. Note the giant cell and the dense cellular reaction.

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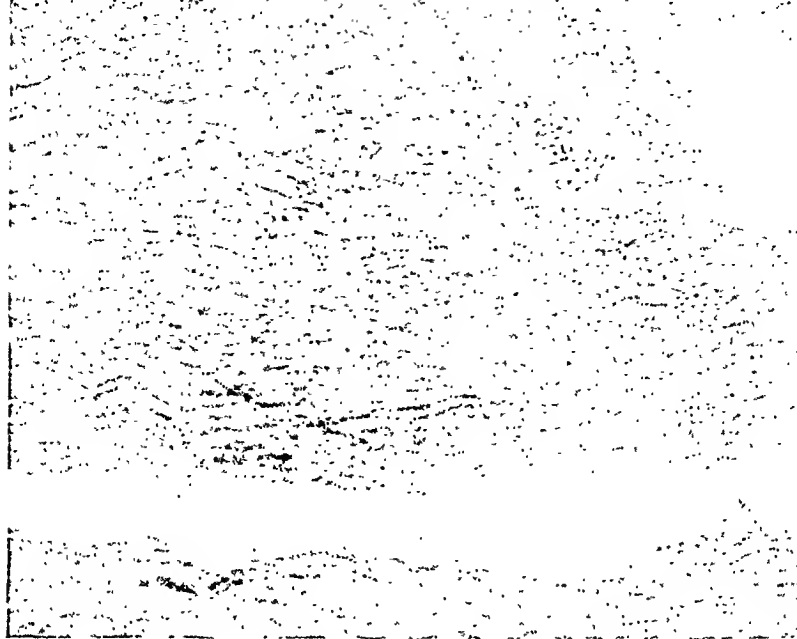


FIG. 6

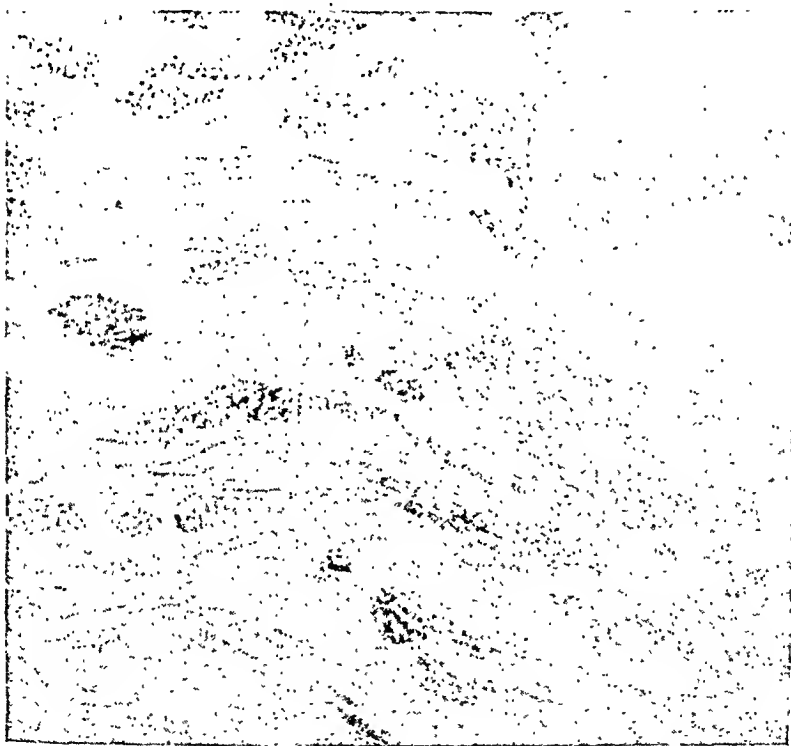


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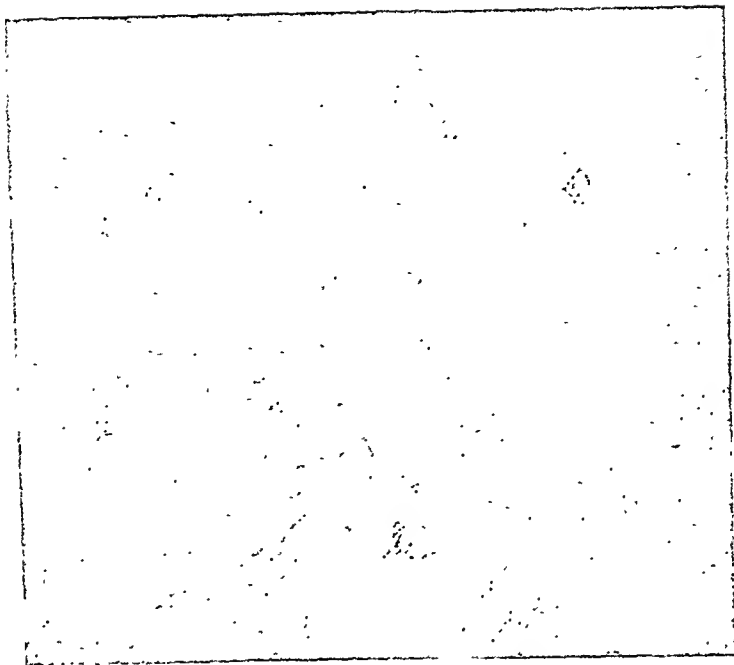


FIG. 4.

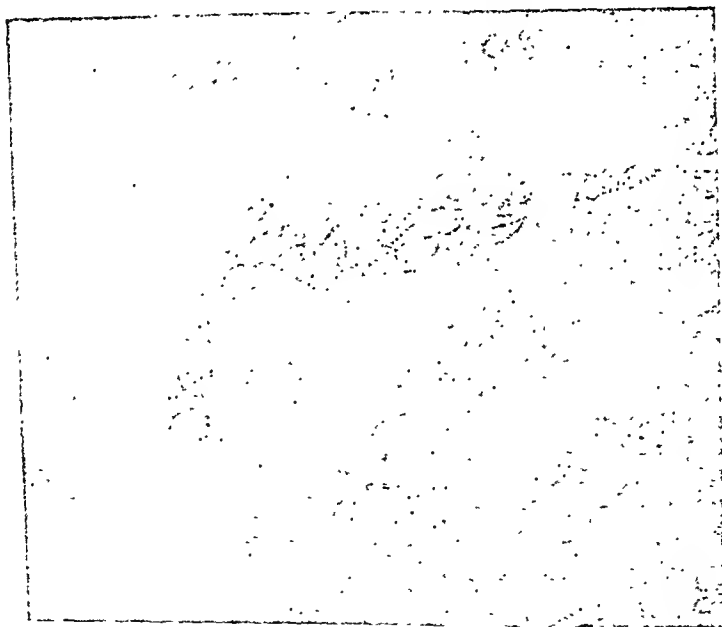


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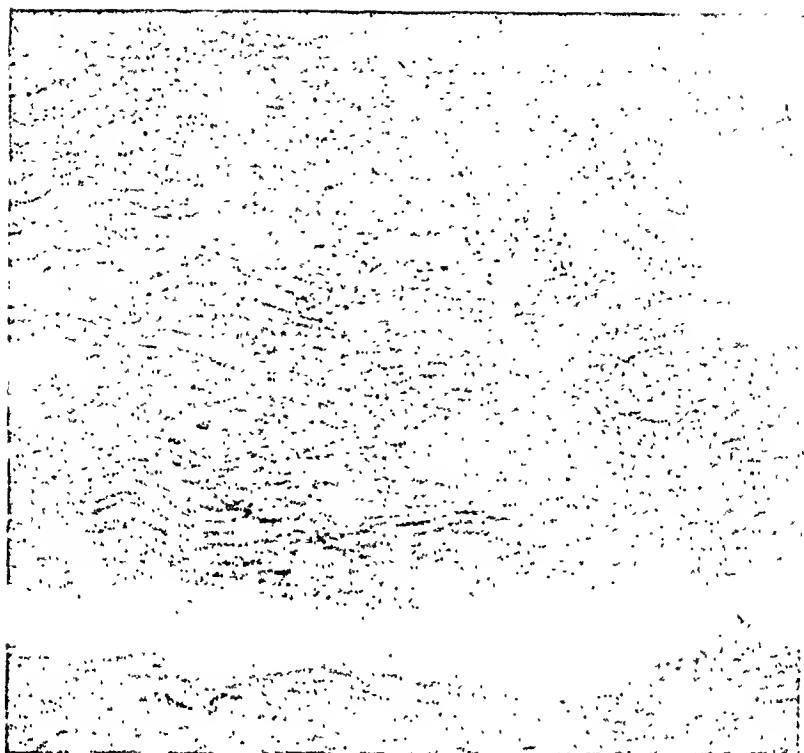


FIG. 6.

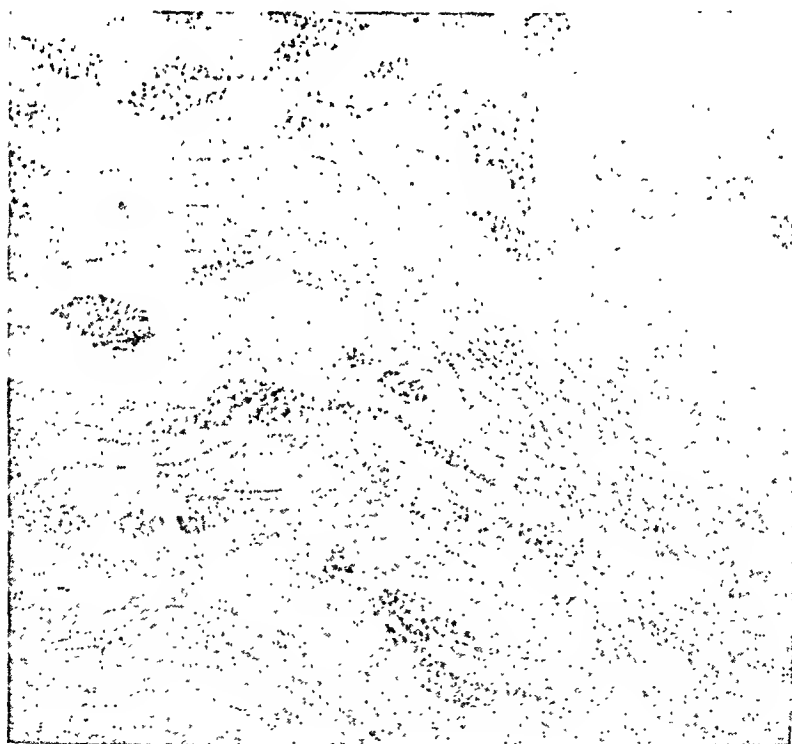


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FIG. 7.

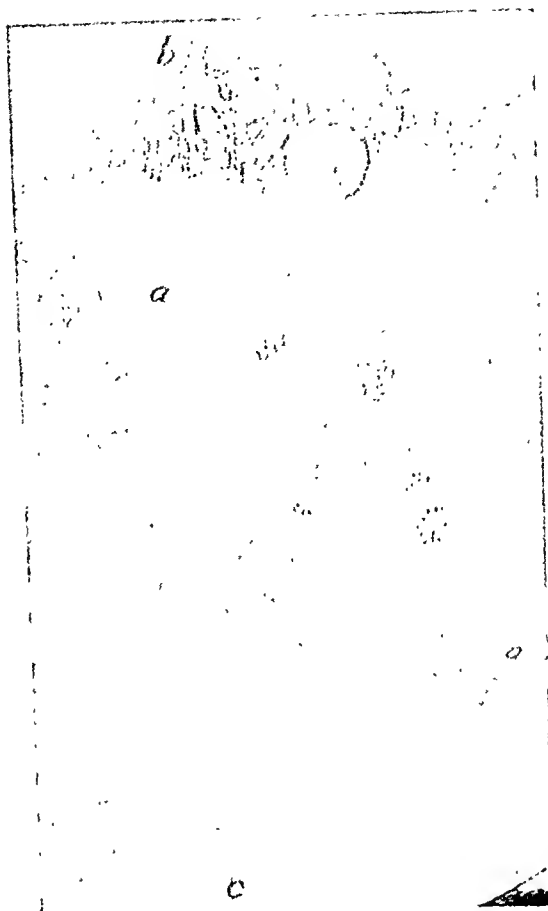


FIG. 8.



FIG. 7.—Section of superficial part of skin in a new lesion in a patient with low resistance due to massive general infection; "a" indicates a papillary vessel with numerous bacilli, intra- and extra-cellular, but no signs of tissue reaction; "b" = epithelium, "c" = hair follicle.

FIG. 8.—Teased nerve fibres in a resistant (N_1) case. Bacilli are seen lying on the nerve fibres. The cells are those of Schwann's sheath. There is no sign of tissue reaction as the fibres were from the superficial part of the nerve bundle.

cause of this susceptibility is supposed to be that the child in a home infected with tuberculosis is liable to receive a greater degree of infection than the adult similarly placed. While increased liability to infection may partly explain the greater susceptibility of children (as compared with adults) to leprous infection, there is strong evidence that this is not the only cause.

When a suspension of killed *B. leprae* is injected intradermally in very young non-leprous children (leprolin test), it produces but little local cellular reaction as compared with a similar inoculation in older children or in adults. This suggests that the defensive mechanism against *B. leprae*, *viz.*, strong cellular reaction resulting in phagocytosis and destruction of bacilli, is weaker in young children than in adults.

The typical form of leprosy found in children who have been in close contact with highly infectious cases during their first years of life is of a peculiar nature. The skin lesions take the form of hyperpigmented macules with slight parakeratosis but without any clearly defined margin. Clinically these lesions are difficult to recognise, while histologically they show only a mild degree of cellular reaction, and but few bacilli. These lesions may appear and disappear from time to time, and they are often widespread over the body. It has been shown that these macules often develop into frank lesions in later life. "Juvenile leprosy" is a convenient term to apply to this type. In adults the early lesions tend as a rule to be more marked clinically and histologically, and they show a greater degree of cellular reaction.

We propound the hypothesis that effective cellular reaction to *B. leprae*, resulting not only in their ingestion but in their destruction, is the main protection of the body against the invasion and spread of these organisms. If this hypothesis is accepted there is considerable evidence that the greater susceptibility of children to leprosy is connected with weaker cellular response to *B. leprae* during the first few years of life, as shown by the weak cellular reaction to inoculated *B. leprae*, and by the type of lesions found in these cases.

(2) *General health*.—A similar weakening of cell activity occurs when the general health is depressed by predisposing or intercurrent diseases, or by any other cause. This, as in juvenile leprosy, is shown by the leprolin test, which shows a milder local reaction to injected bacillary suspension. It is also shown by the clinical and histological examination of lesions. The onset of diseases like malaria, kala-azar, dysentery and enteric is often followed by the flattening out or even disappearance of prominent leprous lesions, due largely to the diminution or cessation of cellular reaction to *B. leprae* present in the skin. The patient may appear to improve as far as his leprous lesions are concerned, while all the time the infection is increasing, uncontrolled by phagocytosis. When convalescence sets in and the general health again improves, cellular reaction is restored and the lesions appear considerably enlarged and more numerous as compared with their condition before the temporary lowering of general health.

(3) *Small infections*.—There is considerable evidence that, as in tuberculosis, small infections with leprosy in otherwise healthy subjects increase the resistance to the disease. This resistance shows itself in enhanced cellular reaction of the endothelial cells to bacilli in their neighbourhood. In such cases the tuberculoid lesion, with epithelioid and giant cells and but few bacilli, is common. In these cases the leprolin test gives a strong reaction, a large nodule, often with central necrosis, forming at the site of inoculation as the result of heightened cellular response to the injected bacilli. If this nodule is removed by biopsy and sectioned, an intense cellular reaction is found with giant cells present; in fact, a lesion similar in appearance to the leprosy lesions present in the skin. In my experience in Northern India the majority of all cases of leprosy are of this type. This is shown to be the case when a careful house to house survey is carried out in endemic areas, though the proportion may be much smaller if those attending a clinic or segregated in an institution be examined.

(4) *Gross infection*.—In contrast to the above, massive infection with *B. leprae*, or the presence of large numbers of these bacilli due to multiplication in the body (see Fig. 7), results in lowered cellular reactivity. When more than a certain number of bacilli have accumulated in the body a state of symbiosis is established between the endothelial cells and the bacilli, the latter being ingested and multiplying in the cells, which gradually become enlarged, vacuolated and destroyed. When the leprolin test is performed in patients with massive infection the result is generally negative, or only very slightly positive. In performing the test a piece of skin may be chosen where the infection is absent or only slight, and into this skin is injected an emulsion rich in *B. leprae* which has been heated to 120° C. for half an hour. If 2 or 3 weeks later the skin at the point of inoculation is removed by biopsy and sectioned, few bacilli are found present in the tissues, and only a mild degree of cellular reaction is seen. The bacilli, not having been ingested by the cells, pass up the lymphatics. In this case, too, the appearance of the leprolin nodule is similar to the lesions of short duration present in the skin.

We have thus four factors, three of which (*viz.*, tender age, debility and massive infection) diminish cellular reactivity to *B. leprae*, and one of which (*viz.*, slight infections) increases their reactivity. These factors may counteract, supplement or succeed each other. Thus temporary depression of general health may counteract for the time-being the increased resistance which has been acquired through small infections; but the state of increased resistance may be restored if the depression of health be not too severe or prolonged. The susceptibility of the first few years of life may be accompanied or succeeded by the low resistance attendant on a high degree of infection, and both of these may be supplemented by weak general health.

The ebb and flow and the interplay of these various factors and their effect

on cellular reaction in the skin and nerves, account to a large extent for the multiformity of leprous lesions, so difficult to describe and explain.

So far we have referred chiefly to skin lesions, but the same laws and the same factors hold good in the peripheral nerves. When the skin is infected the infection not only spreads in the skin, but also passes up the supplying nerve branches. In fact, there is reason to believe that under certain circumstances the nerves form a more fertile medium than the skin for the multiplication of bacilli. This is apparently due, at least in part, to the comparative paucity of endothelial phagocytic cells in the nerves as compared to the skin. The bacilli enter a nerve bundle and pass up it in the spaces between the nerve fibres (see Figs. 5 and 6), and are thus not in close contact with the endothelial cells of the vascular capillary in the centre of the bundle. Sections of nerve bundles often show cellular multiplication and destruction of the central nerve fibres; while at the periphery of the bundle cellular proliferation is absent, the bacilli are present in large numbers and the nerve fibres remain intact.

It is questionable if the skin is ever infected to any marked extent without the supplying nerves becoming infected too. We speak of skin leprosy and nerve leprosy, and in the former it may be impossible to find clinical evidence of nerve affection. But in skin leprosy microscopic examination of the nerves shows the presence of large numbers of bacilli. In this type of the disease the absence of clinical signs connected with the nerves is not due to the absence of bacilli in their bundles, but to the comparative weakness of cellular response to the bacilli which are lying between the nerve fibres. The swelling and tenderness of nerves and the sensory and trophic changes in their distribution are due not to toxins set free from the bacilli, nor to pressure of the bacilli on the nerve fibres; they are due to the mechanical pressure of the cellular granuloma, *i.e.*, of the cells proliferating in response to the bacilli lying between the nerve fibres. Thus, though in the low resistant case there is massive infection of the nerve, the absence of marked cellular response accounts for the fact that neural signs are comparatively slight.

In the more resistant case, however, bacilli may be few or absent in the skin, having been destroyed by the strong phagocytic cellular response; at the same time the nerves are swollen and tender, the degree of resistance being sufficient to stimulate the endothelial cells of the central capillary of the nerve bundle to high activity against the bacilli present between the nerve fibres (see Figs. 2, 3, 4, 8). This can be demonstrated by making simultaneous clinical and histological studies.

Many theories have been advanced for the curious fact that neural signs and symptoms are so slight in cases in which bacilli are numerous in the skin, and so marked in cases in which the bacilli are comparatively few in the skin. The above, if confirmed by other workers, would seem to offer a satisfactory explanation of this phenomenon.

Effects of varying resistance.—We have mentioned above that not only do

(3) *Small infections*.—There is considerable evidence that, as in tuberculosis, small infections with leprosy in otherwise healthy subjects increase the resistance to the disease. This resistance shows itself in enhanced cellular reaction of the endothelial cells to bacilli in their neighbourhood. In such cases the tuberculoid lesion, with epithelioid and giant cells and but few bacilli, is common. In these cases the leprolin test gives a strong reaction, a large nodule, often with central necrosis, forming at the site of inoculation as the result of heightened cellular response to the injected bacilli. If this nodule is removed by biopsy and sectioned, an intense cellular reaction is found with giant cells present; in fact, a lesion similar in appearance to the leprous lesions present in the skin. In my experience in Northern India the majority of all cases of leprosy are of this type. This is shown to be the case when a careful house to house survey is carried out in endemic areas, though the proportion may be much smaller if those attending a clinic or segregated in an institution be examined.

(4) *Gross infection*.—In contrast to the above, massive infection with *B. leprae*, or the presence of large numbers of these bacilli due to multiplication in the body (see Fig. 7), results in lowered cellular reactivity. When more than a certain number of bacilli have accumulated in the body a state of symbiosis is established between the endothelial cells and the bacilli, the latter being ingested and multiplying in the cells, which gradually become enlarged, vacuolated and destroyed. When the leprolin test is performed in patients with massive infection the result is generally negative, or only very slightly positive. In performing the test a piece of skin may be chosen where the infection is absent or only slight, and into this skin is injected an emulsion rich in *B. leprae* which has been heated to 120° C. for half an hour. If 2 or 3 weeks later the skin at the point of inoculation is removed by biopsy and sectioned, few bacilli are found present in the tissues, and only a mild degree of cellular reaction is seen. The bacilli, not having been ingested by the cells, pass up the lymphatics. In this case, too, the appearance of the leprolin nodule is similar to the lesions of short duration present in the skin.

We have thus four factors, three of which (*viz.*, tender age, debility and massive infection) diminish cellular reactivity to *B. leprae*, and one of which (*viz.*, slight infections) increases their reactivity. These factors may counteract, supplement or succeed each other. Thus temporary depression of general health may counteract for the time-being the increased resistance which has been acquired through small infections; but the state of increased resistance may be restored if the depression of health be not too severe or prolonged. The susceptibility of the first few years of life may be accompanied or succeeded by the low resistance attendant on a high degree of infection, and both of these may be supplemented by weak general health.

The ebb and flow and the interplay of these various factors and their effect

IRRITATION CAUSED BY CONTACT WITH THE PROCESSIONARY CATERPILLAR (LARVA OF *THAUMETOPOEA WILKINSONI* TAMS) AND ITS NEST.

BY

R. L. CHEVERTON, M.R.C.S., L.R.C.P.,

*Senior Medical Officer, Falkland Islands ; sometime District Medical Officer, Cyprus.**

The processionary moth belongs to the order Lepidoptera and is found in Europe, Africa, Madagascar and Cyprus.

Together with other caterpillars of this division it has the property, on coming into contact with the skin or mucous membrane, of causing great irritation, as does also the dust from the nest which is a dense web, shaped like a conical bag usually $\frac{1}{2}$ to 1 foot in length.

In Cyprus, *Thaumetopoca wilkinsoni* Tams, a lepidopteran, is found on all species of pine, *Pinus halepensis* Mill being usually the one specially selected by this caterpillar.

The life history of the processionary caterpillar (Fig. 1) is briefly this : The adult emerges during the period August to October, there are five larval instars, the eggs being laid in October. Pupation takes place in the ground in silk cocoons during March at sea level, but at the higher altitudes the adult emerges a little earlier owing to the cooler weather.

The nest, as found in Cyprus, varies from 4 to 12 inches in length, and is conical in shape with its apex hanging free (Fig. 2). The colour when new is white and glistening but later turns to a dull brown. The nest appears in the small pine copses, particularly on the younger trees while the more mature trees remain free from assault. It has been observed that the pine trees at higher altitudes of 2,000 to 4,500 feet (beyond which height the caterpillar is not

* My thanks are due to Mr. W. MORRIS, Government Entomologist, Cyprus, for so kindly taking the photograph of the larva and the photomicrograph of the hair ; also to Professor F. S. BODENHEIMER, Professor of Zoology, the Hebrew University, Jerusalem, for his helpful communication.

could be kept only about a month, it is now found practicable to make these up in quantity two or three times during the year, as convenience dictates ; and to keep them apparently indefinitely without any visible deterioration. A comparison of samples of the same batch of medium tubed in the old way and in the new in a short time affords a striking demonstration of the superiority of the latter. With liquid media a similar result has been obtained, and the very obvious diminution in bulk of the fluid, at one time so constant a feature due to evaporation after about a week, has been entirely obviated. In the case of the carbohydrates, McCARTNEY's method of sterilisation of the sugar solutions by filtration through Seitz filters, and their addition to the stock parent medium shortly before use, has proved quite satisfactory and is, moreover, economical in the case of the more expensive sugars.

In this particular colony the departmental laboratory is freely available for use by the large number of private medical practitioners in the island. Blood culture is in frequent demand by them ; and media are supplied at their request, inoculated by them and returned for examination at the laboratory. These specimens arrive by various routes and after varying periods of time after issue. The employment of 6 or 8 ounce bottles provided with perforated caps and rubber washers protected by "viskaps" has materially assisted in eliminating some of the more undesirable features of such a system.

In one respect local experience has not been as happy as that recorded by McCARTNEY. While the smaller bottles and vials, up to 6 or 8 ounces capacity, withstood, with commendable fortitude, the vicissitudes of travel and the subsequent manipulations involved in frequent cleaning and sterilising the larger bottles suffered severely in transit. This could be overcome by adequate packing. The initial breakage-rate with these larger sizes on first autoclaving was and continues to be excessively high in spite of every precaution designed to arrest it. It would appear that once a bottle has withstood its first experience in the autoclave, it can subsequently be similarly treated with comparative impunity. Our experience is that for storing media in quantities over 6 or 8 ounces the bottle with stoneware stopper, clip and rubber washer is preferable as, with reasonably careful handling, it seems immune to fracture. With this exception it has been found that McCARTNEY's technique is particularly suitable for the conditions obtaining in this colony, and it would appear admirably adapted for bacteriological work throughout the tropics in general. From the aspects both of efficiency and of economy, it has proved greatly superior to the older methods of procedure in general use under difficult climatic conditions.

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McCARTNEY, J. E. & AYLING, T. H. (1935). Further notes on the laboratory use of screw-capped bottles. *Ibid.*, i, 1388.

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found) are more prone to be attacked ; though isolated examples are found even at sea level.

From a medical point of view the caterpillar is of interest, as it causes intense irritation when handled or when its nest is disturbed to such an extent as to cause a fine dust to be sprayed out. Contact with this will give rise almost instantaneously to large urticarial wheals, and intense irritation which will last as long as the dust remains on the clothing. Entomologists are advised to use rubber gloves when handling the larvae and their nests ; often the dust collects around the cuffs and will continue to irritate the skin for months every time the same coat is worn. Children playing amongst pine trees have been known to knock these nests with a stick and the dust immediately sets up an acute conjunctivitis.

Though I have not actually seen such a case, it is reported by the villagers in Cyprus that they find great difficulty in swallowing if they have drunk water from a well over which is hanging a pine tree containing a nest.

There are various theories as to this toxic substance but nothing definite has been formulated. By some it is supposed to be formic acid but I am unable to find any authority for this opinion. FABRE believes that the excreta in the nests, with which the caterpillars come into contact, is the toxic agent ; this fact might account for the idea of the peculiar toxic properties of drinking water contaminated by proximity to a nest. MATHESON says that the urticating properties are due to special hairs or spines supplied with poison cells. (Fig. 3.) These hairs are hollow and contain the poison, each hair having a gland cell opening into the seta, which contains the poisonous secretion ; the fine barbed hairs penetrate the surface causing the poison to spread. The secretion inside the seta retains its urticating properties for a long time, thus the hairs retain their toxic effects.

Dr. BODENHEIMER of the Hebrew University, Jerusalem, informs me that FABRE's theory is but part of the truth. The function of the two glands at the base of the hair is still unknown ; it is, however, interesting to note that hairs washed with ether and then placed in the skin do not cause any irritation, but the etheric extract does cause an urticaria. Pure physical irritation of the hair itself may thus be excluded.

Case.

A Cypriot boy reported at the Limassol District Hospital a few hours after he had disturbed a nest in a pine tree near the shore. The conjunctiva was very injected and there was oedema of both eyelids (Fig. 4).

On the following day his right eye was completely closed with oedema and the other partially closed, the oedema had spread over the whole face. After a few days the general condition improved but there appeared a local and superficial gangrene of the right upper and lower eyelid. Gradually this gangrenous area separated, leaving, after 3 weeks, a superficial scar, a corneal ulcer and chronic conjunctivitis with a slight photophobia.

FIG. 2

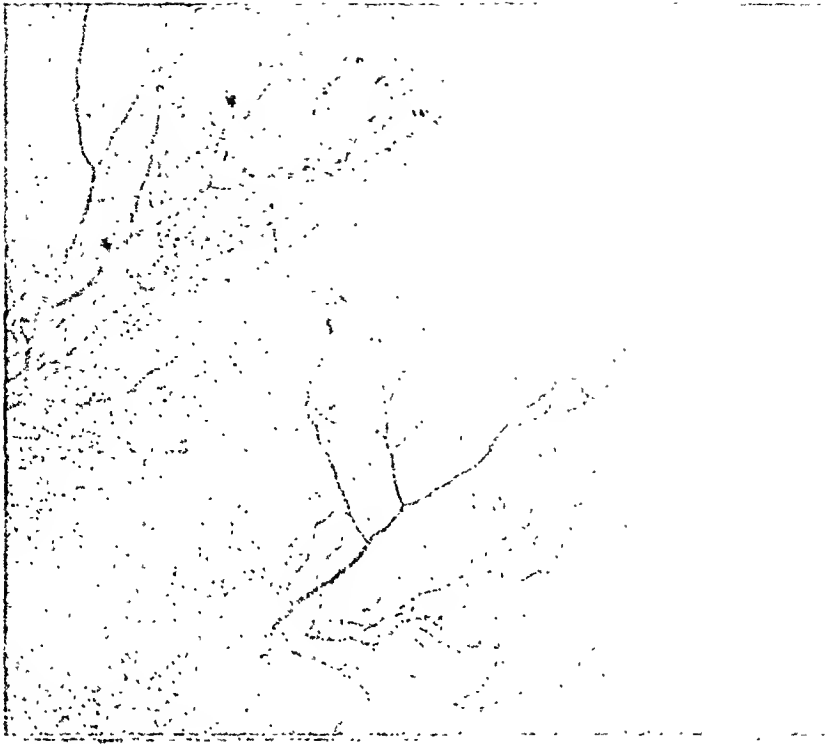


FIG. 1

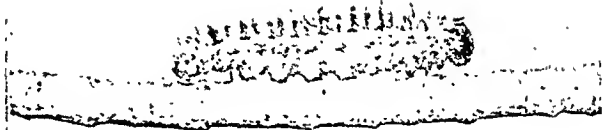


FIG. 3

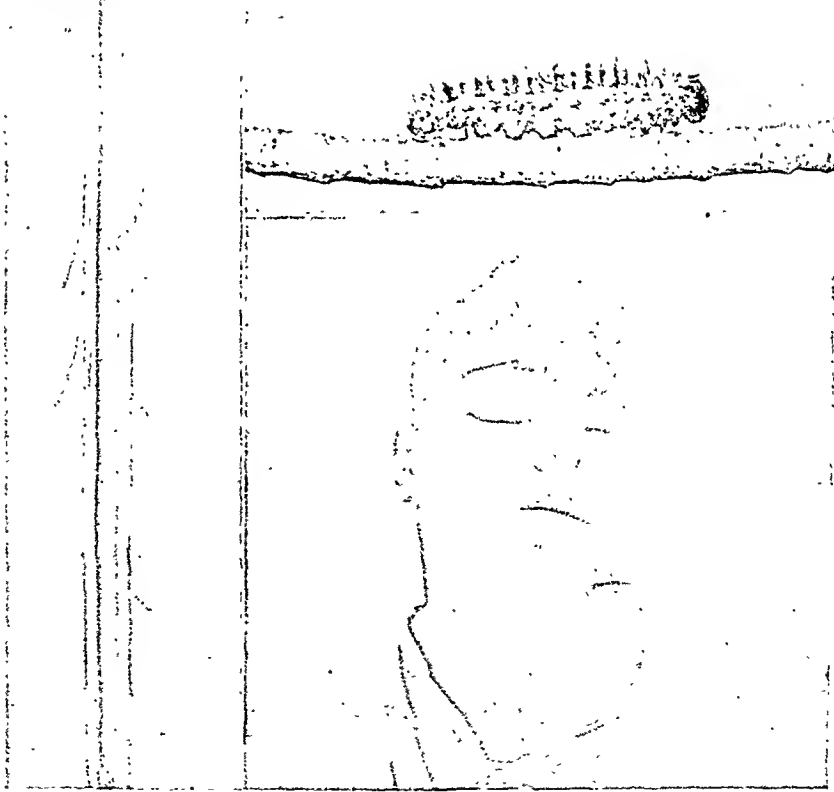
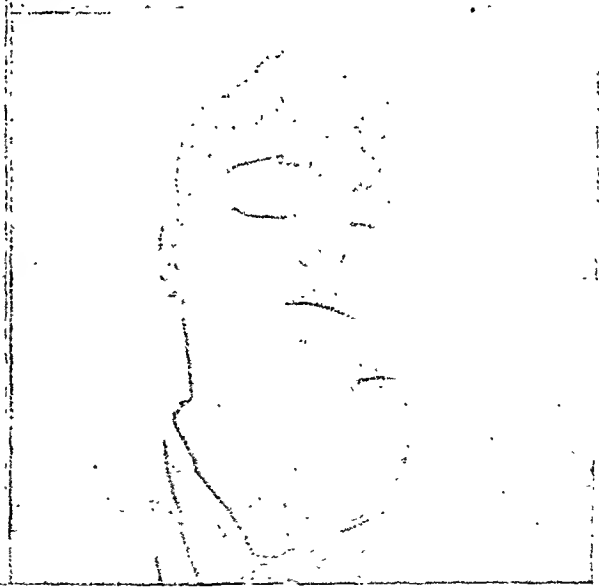


FIG. 4



THE PROCESSIONARY CATERPILLAR. Larva of *Thaumetopoea wilkinsoni* Tams.
Fig. 1.—The caterpillar. Life size. Fig. 2.—Nest in a pine tree. These nests vary in size from 4 to 12 inches. Fig. 3.—Hollow hair of caterpillar. $\times 500$. Fig. 4.—Cypriot boy's face showing intense reaction after contact with a nest.

found) are more prone to be attacked ; though isolated examples are found even at sea level.

From a medical point of view the caterpillar is of interest, as it causes intense irritation when handled or when its nest is disturbed to such an extent as to cause a fine dust to be sprayed out. Contact with this will give rise instantaneously to large urticarial wheals, and intense irritation which lasts as long as the dust remains on the clothing. Entomologists are advised to wear rubber gloves when handling the larvae and their nests ; often the dust around the cuffs and will continue to irritate the skin for months after the same coat is worn. Children playing amongst pine trees have been known to knock these nests with a stick and the dust immediately causes conjunctivitis.

Though I have not actually seen such a case, it is reported from Cyprus that they find great difficulty in swallowing if they are near a well over which is hanging a pine tree containing a nest.

There are various theories as to this toxic substance which has been formulated. By some it is supposed to be formed by the caterpillars themselves. FABRE believes that the dust with which the caterpillars come into contact, is the cause of the irritation. He accounts for the idea of the peculiar toxic dust by supposing that the caterpillars are contaminated by proximity to a nest. MATTHEWS believes that the properties are due to special hairs or spines situated on the caterpillars. These hairs are hollow and contain the poison. The opening into the seta, which contains the poison, is situated at the base of the hair. The hairs penetrate the surface causing the poisoning. The seta retains its urticating properties and causes the same toxic effects.

Dr. BODENHEIMER of the Hebrew University, Jerusalem, states that FABRE's theory is but part of the truth. The base of the hair is still unknown. He states that a piece of skin washed with ether and then placed in the etheric extract does cause the same effects. The etheric extract itself may thus be excluded.

A Cypriot boy reported that he had disturbed a nest of the processionary caterpillar. He was injected and there was a severe reaction.

On the following day the boy was again injected and the other partial injections. A few days later the boy had a superficial gangrenous area on his arm. The gangrenous area was removed and the ulcer and chronic inflammation.

Hot fomentations of sodium bicarbonate and later the application of 2 per cent. ichthyol were used. MÖNNIG advocates the application of ammonia to the skin followed by 10 per cent. ichthyol.

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CORRESPONDENCE.

HUMAN BRUCELLOSIS IN TANGANYIKA TERRITORY.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

DEAR SIR,

In a communication on this subject by D. E. WILSON in these TRANSACTIONS, Vol. XXIX, No. 4, p. 366, the author states that "MANSON-BAHR (1935) [these TRANSACTIONS, XXVIII (4), 374 (Discussion)] has reported favourably on the intravenous use of T.A.B. vaccine as a means of increasing the euglobulin content of the serum and thus promoting phagocytosis of the offending *Brucella*."

We would like to point out that while MANSON-BAHR has drawn attention to the efficacy of T.A.B. vaccine in this disease this possible explanation of its mode of action was suggested by us in these TRANSACTIONS, 1935, Vol. XXVIII, p. 370.

' We are, etc.,

*The Wellcome Bureau of Scientific Research,
London.
10th February, 1936.*

H. C. BROWN,
J. C. BROOM.

LYMPHOSTATIC VERRUCOSIS.

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.

DEAR SIR,

I read with interest Dr. MANUWA's report of a case of "lymphostatic verrucosis,"* and should like to add that this title was not selected without due forethought, for the introduction of a new name into an already unwieldy terminology is not an operation to be undertaken lightheartedly.

I had previously considered "elephantiasis verrucosa" and rejected the name because a number of cases had been seen in which the verrucosity was secondary to renal oedema and other conditions not strictly falling within the definition of elephantiasis. I fully agree with Dr. MANUWA that there is an "undoubted association of the disease with elephantiasis," but I fear that he has overlooked the qualification that a few cases are not associated with elephantiasis, when he proposes reviving the title of "elephantiasis verrucosa."

I am, etc.,

Soroti, Uganda.

31st December, 1935.

L. J. A. LOEWENTHAL.

* MANUWA, S. L. A. (1935). Lymphostatic verrucosis. *Trans. Roy. Soc. Trop. Med. & Hyg.*, xxix, 289.

TRANSACTIONS
OF THE
ROYAL SOCIETY OF TROPICAL MEDICINE
AND HYGIENE.

VOL. XXIX. No. 6. APRIL, 1936.

Proceedings of an **Ordinary Meeting** of the Society, held at
Manson House, 26, Portland Place, W.1, at 8.15 p.m.,
on Thursday, 20th February, 1936.

Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

The President : Before we come to the business of the meeting I must refer to the great loss this Society has sustained through the death of KING GEORGE V who had been our Patron since December, 1923.

I will read the letter which has been sent from the Society to HIS MAJESTY KING EDWARD ; and ask you to stand in silence as an expression of your homage and respect.

TO THE
KING'S MOST EXCELLENT MAJESTY.

We, the Fellows of the Royal Society of Tropical Medicine and Hygiene, beg leave to express our profound sympathy with YOUR MAJESTY, with QUEEN MARY, and with the other Members of the Royal Family on the death of KING GEORGE V our most gracious and beloved PATRON.

Confident in the knowledge of YOUR MAJESTY's unfailing interest in the welfare of the Tropics and the work of our Society we, the Fellows of the Royal Society of Tropical Medicine and Hygiene, humbly tender to YOUR MAJESTY on your Accession to the Throne our heartfelt and loyal good wishes for a Reign long, peaceful and prosperous.

Signed on behalf of the Fellows of the
Royal Society of Tropical Medicine and Hygiene,

ARTHUR G. BAGSHAWE,
President.

PAPER.

RECENT WORK ON THE TYPHUS-LIKE FEVERS OF MALAYA.

BY

R. LEWTHWAITE*

AND

S. R. SAVOOR,

From the Division of Pathology, Institute for Medical Research, Kuala Lumpur, F.M.S.

Although the typhus-like fevers do not assume the importance in Malaya of such diseases as pulmonary tuberculosis, malaria, lobar pneumonia, and dysentery, certain of their features have compelled the attention of workers in that country. The recognition of the rural† form of tropical typhus by FLETCHER and LESSLAR in 1924, was followed at once by the recognition of a second and third form of typhus fever, the urban typhus and the tsutsugamushi disease. The lethality is as high as that of lobar pneumonia in England. Rural

* In addition to acknowledgements made in the text, the authors wish to thank the following of their colleagues at the Institute for Medical Research: Dr. A. NEAVE KINGSBURY, Director, for his interest in these investigations; Dr. P. H. MARTIN, sometime Bacteriologist, Dr. R. GREEN, his successor, and Dr. D. S. MANKIKAR, Assistant Bacteriologist, for their ready help in carrying out the large number of Weil-Felix reactions that were required.

† The rural form of tropical typhus is also referred to in the literature as scrub-typhus, and the urban form as shop-typhus. The first designation will be adhered to in this paper, and for brevity will be written as rural typhus and urban typhus respectively.

typhus and the tsutsugamushi disease are predominantly diseases of the planting community ; many of the fatal cases have been in men of fine physique in the early twenties, so that the appearance of these diseases has aroused much perturbation and speculation as to the threat entailed to the welfare of this important community. Would the diseases spread and devastate ?

The researches of FLETCHER and his collaborators (see references), of GATER (1930), and of ANIGSTEIN (1933), dispelled much of the dread that these fevers inspired. Thus, though clinically all greatly resembled the classical typhus exanthematicus, they were shown to differ from it entirely in epidemiology, and two of them in serology. Lice played no part in transmission ; the diseases were endemic, not epidemic ; a period of economic distress would not entail decimation of populations, as the experience of the extreme depression of 1931-33 was later to prove. Evidence was obtained that strongly suggested that *Trombicula akamushi* and *T. deliensis* were the vectors of rural typhus and the tsutsugamushi disease ; and, further, that these two larval trombidids are probably merely forms of the same species. Investigations also indicated that the viruses of these two diseases shared the only reservoir yet known—the rat ; and that the causal organism of rural typhus was rickettsial in its nature. Attempts to establish and maintain strains of the viruses of rural typhus and the tsutsugamushi disease in laboratory animals were frustrated by the insusceptibility of the guineapig, rat, and rabbit.

SCOPE OF RECENT RESEARCH.

The work to be reported is, broadly, a continuation of the various lines of investigation indicated in the above summary ; and has been carried out during the last four years. The major results obtained can be grouped under five headings, *viz.* :—

1. Demonstration of the rickettsial nature of the viruses of all three members of the tropical typhus group.
2. The relationship of the three diseases to each other and to Rocky Mountain spotted fever.
3. Search for the carriers of rural and urban typhus.
4. Evidence as to the reservoir of the viruses.
5. Attempted vaccination of guineapigs against experimental infection with rural typhus.

(In this paper the tsutsugamushi disease and rural typhus are referred to as separate entities in virtue of the clinical distinction met with in human cases, *viz.*, the occurrence of an initial ulcer and bubo in the former, and their absence in the latter.)

Little progress was made, or could be made, until the viruses could be studied under experimental conditions in the laboratory. The development of all these five lines of research was the direct outcome of the initiation and maintenance of strains of the three typhus-like fevers in laboratory animals.

*THE EXPERIMENTAL INFECTION OF LABORATORY ANIMALS.

THE GUINEAPIG.

Rural Typhus.—Upwards of 250 guineapigs had been inoculated with blood drawn from 100 or more patients early in the course of the disease by the authors and by ANIGSTEIN, with little success. Febrile reaction was in general absent and once only could a strain be maintained as far as the 12th generation. It was therefore had to the use of vitamin-deficient guineapigs, which were used some days before and after inoculation on a diet of water and autoclaved milk and rolled oats, a diet reported on favourably by ZINNSER, C. and SEASTONE (1931), as enhancing the virulence of experimental infection with the endemic typhus of the United States of America. Again no reaction ensued, save for one notable exception. From one patient (Seerangayee) a strain was initiated, maintained precariously for the first few generations, but grew more easily, until eventually the use of the vitamin-deficient diet became necessary. This strain, called the "Seerangayee" strain, has been maintained for 4 years without loss of virulence. The inoculum is usually brain or, less often heart-blood, splenic tissue, or ascitic fluid, injected intra-peritoneally. The clinical signs in guineapigs infected by this route are an incubation period of 7 to 12 days, followed by continuous fever of 7 to 9 days' duration, and scrotal swelling occurs; 90 per cent. of guineapigs infected by this route recover.

Constant postmortem signs are ascites and enlargement of the spleen. *Rickettsia* are found with ease and in abundance in the ascitic fluid and, usually, in the fibrinous deposit usually present on the surface of the spleen. *Rickettsia* nodes are to be found in sections of the brain, but only rarely. Guinea-pigs that recover are immune for at least 15 months.

The Tsutsugamushi Disease.—Attempts to maintain strains of this disease in guineapigs have all failed, though they have been few in number through lack of opportunity. One strain was maintained for nine generations by the use of guineapigs, and then lost; in those animals that reacted, fever, enlargement of the spleen, and ascites were present, and *rickettsia* indistinguishable from those demonstrable in experimental rural typhus were found in the ascitic fluid and fibrin on the spleen.

Thus rural typhus and the tsutsugamushi disease are alike both in the marked insusceptibility of the guineapig to the respective viruses, and in the lack of the criteria of infection when such be secured.

Urban Typhus.—The first attempt to infect guineapigs with the urban typhus was successful. Blood was drawn from a patient on the height of fever, and inoculated intra-peritoneally into two guineapigs. Both animals died with fever and a redness, tenderness, and swelling of the scrotum. By intra-peritoneal inoculation of brain, blood, or exudate from the tunica vaginalis.

* A detailed description of these experimental infections, with drawings, photographs, and photomicrographs, has recently been published (LEWTHWAITE and SAVOOR, 1932). They are therefore here described only in brief.

strain has been maintained in guineapigs for $3\frac{1}{2}$ years without loss of virulence. The incubation period is 5 to 7 days if the inoculum be tunica exudate, 6 to 9 days otherwise. The febrile reaction lasts for 6 to 7 days. When the route of inoculation is intra-peritoneal, the scrotal reaction occurs in 95 per cent. of male guineapigs if the inoculum be tunica exudate, and in 70 per cent. if the inoculum be brain, splenic tissue or heart-blood. It never follows subcutaneous inoculation. Its onset usually coincides with the onset of fever. Reaching the maximum degree of intensity on the second day, it is gone by the 5th day; exceptionally, its course may be as short as 24 hours. Death is very rare. Infection confers an immunity of at least 6 months' duration.

The only constant postmortem signs are enlargement of the spleen and the marked changes of the scrotal reaction. In contrast to the corresponding findings in rural typhus, the splenic surface is smooth and ascites absent. The changes that constitute the scrotal reaction are a swelling of the testis, an injection of the two layers of the tunica vaginalis, both of which are moist with an exudate that becomes more fibrinous and gluey as the condition advances, a haemorrhagic condition and gelatinous consistence of the polar fat, and oedema of the scrotal tissues. Smears of the exudate show it to be predominantly polymorphonuclear in the early stages of the reaction, mononuclear in the later stages. Rickettsia are present within the cytoplasm of the endothelial cells that lie in the exudate.

THE RABBIT.

Rural Typhus.—Intra-peritoneal inoculation of infected human blood or of passage virus into rabbits results in the development of a positive Weil-Felix reaction in about one-half the number of cases; the titres are low. Agglutination is of the OXK type of *Proteus* X strains. No other sign of infection is evident. Attempts to establish strains by intra-testicular inoculation of virus failed.

In view of these meagre results and of the marked insusceptibility of the guineapig, the method of intra-ocular inoculation of rabbits, practised by NAGAYO *et al.* (1931) in the case of the tsutsugamushi disease of Japan, and typhus exanthematicus, was tried by the authors; and was at once successful. Inoculations of blood by this route from four of twelve human cases seen early in the course of their disease resulted in the initiation of strains. The guineapig strain (Seerangayee) of rural typhus can be transferred at will to rabbits by the intra-ocular inoculation of heart-blood drawn at the height of febrile reaction.

The signs of infection are an intense irido-cyclitis in the inoculated eye, the development of agglutinins in the rabbit's serum for the OXK type of *Proteus* X strains, and the presence, readily demonstrable, of the causal rickettsia in the cells of the endothelium covering Descemet's membrane at the back of the cornea. The details of technique, evolution of the irido-cyclitis, and the

demonstration of rickettsia, have already been described by the authors (LEWTHWAITE and SAVOOR, 1936). Later passages are secured by the intra-ocular inoculation of infected aqueous humour, drawn at the height of the ocular reaction. Once the first two or three passages are successfully effected, there is no difficulty in the maintenance of strains; fully 99 per cent. of rabbits react. Five strains of rural typhus were maintained sufficiently long to enable cross-immunity tests to be made, and were then discarded. The condition of the rabbits remains good throughout the infection, and all recover. Fever is insignificant or absent altogether. After convalescence the rabbits are immune for a period of at least 12 months.

The Tsutsugamushi Disease.—The intra-ocular method was successful also in the case of the tsutsugamushi disease. From two of three patients seen early in the course of the disease inoculation of blood resulted in the initiation of strains that were maintained with ease once the second and third passages were secured. One strain, the Raub strain, has been thus maintained for $3\frac{1}{2}$ years. Another strain, the Kepong strain, was maintained for the few generations requisite for the completion of cross-immunity tests. The signs of infection differed in no way from those typical of experimental rural typhus. Thus the irido-cyclitis is the same in evolution and intensity, the rabbit after convalescence is healthy and immune, rickettsia that appear identical with those of rural typhus are readily demonstrable in smears from the infected eye; while the serum develops agglutinins to the OXK type of *Proteus* X strains.

Urban Typhus.—Intra-peritoneal inoculation of human blood or passage virus into rabbits results in the development of a positive Weil-Felix reaction, in approximately one-half of the cases, agglutination being of the OX19 type of *Proteus* X strains; no other evidence of infection has been observed.

A large number of attempts have been made to establish in rabbits the guineapig strain of urban typhus by the intra-ocular injection of virus. The results have differed considerably from those reported above in the case of rural typhus and the tsutsugamushi disease. Inocula of infected human or guineapig blood, of tunica exudate from infected guineapigs, or of emulsions of infected fleas (*Xenopsylla cheopis*), all of which inocula were proved infected by inoculation of control guineapigs, have evoked an ocular reaction in 59 per cent. of rabbits. Attempts to maintain strains thus initiated, by inoculation of infected aqueous humour, have yielded only 32 per cent. successes in the second generation, 18 per cent. in the third, and nil in the fourth. Infected rabbits show no generalized ill-effects. The reaction differed from those caused by the virus of rural typhus and the tsutsugamushi disease only in duration and intensity, being shorter and milder.

The inability to maintain strains of urban typhus beyond three generations contrasts markedly with the ease of maintenance of the viruses of rural typhus and tsutsugamushi. It corresponds closely, however, with the results of NAGAYO *et al.* (1930) in their study of the virus of typhus exanthematicus in rabbits

infected by the intra-ocular method ; and with those obtained by the authors in the case of the virus of Rocky Mountain spotted fever.

Rickettsia can be demonstrated within the cells of the endothelium covering Descemet's membrane, but they are scanty. A positive Weil-Felix reaction developed in 52 per cent. of rabbits inoculated by the intra-ocular route, titres as high as 1/1925 being recorded ; agglutination was of the OX19 type of *Proteus* strains, never of the OXK type.

THE WHITE RAT.

Rural Typhus.—The attempts made to infect white rats had a twofold object in view ; firstly, to apply to the virus the criterion of NICOLLE (1933), *viz.*, that the virus of a murine typhus strain could be maintained indefinitely in the white rat, whereas the virus of Old World typhus could not ; and secondly, to secure a scrotal reaction, and thereby a richer supply of rickettsia, in the guinea-pigs, of the Seerangayee strain of rural typhus by the interpolation of an infected rat in the series of guineapig passages, a procedure which had been successful in the hands of PINKERTON (1931) in the case of a strain of Old World typhus.

Infection was readily secured, but was "inapparente" in type ; fever was insignificant or absent, and serotal reaction did not occur. Inoculation of tissue virus from an infected rat into a guineapig always gave an infection of high virulence. The majority of rats died of the infection. At postmortem, enlargement of the spleen was always present, ascites usually and, occasionally, a fibrinous deposit on the spleen in which rickettsia could be found. Propagation of the strain for 21 generations was effected, and then discontinued. Thus, by NICOLLE's criterion, the virus of rural typhus is murine in type.

Urban Typhus.—Only some twenty inoculations have been made. Infection, readily secured, has always been "inapparente," apart from one dubious scrotal reaction. The rats survive infection. Inoculation of tissue virus from an infected rat into a guineapig by the intra-peritoneal route causes a most intense infection.

THE MONKEY.

The inoculations described below were made to obtain experimental evidence regarding the clinical distinction between rural typhus and the tsutsugamushi disease in man, *viz.*, the presence of the initial ulcer in the latter and its absence in the former.

Rural Typhus.—One gibbon and three macacus monkeys were inoculated by the intra-dermal route. For two of them the inoculum was ascitic fluid and fibrin from the spleen of reacting guineapigs of the Seerangayee strain ; for the other two it was infected material from the eyes of rabbits of the same strain, which strain had been transferred from guineapig to rabbit by intra-ocular

inoculation of infected heart blood. Infection resulted in all four animals, marked by fever, leucopenia, a positive Weil-Felix reaction, and, especially, by dermal lesions at the sites of inoculation, which lesions in all animals passed through a macular and papular stage to end as the small circumscribed ulcers, each with black necrotic centre and surrounding hyperaemic areola, that constitute the initial lesion of the tsutsugamushi disease in man. In three of the four animals swelling of the lymphatic glands also developed.

The Tsutsugamushi Disease.—One gibbon and one macacus monkey were inoculated with infected material from the eyes of rabbits of the Raub strain. Infection occurred in both, marked by precisely the same features as described above in the case of experimental rural typhus. The dermal lesions in the gibbon progressed only to the papular stage, but to the necrotic stage in the macacus.

Urban Typhus.—One gibbon and two macacus monkeys were inoculated intra-dermally with exudate from the tunica vaginalis of reacting guineapigs of the Manickam strain. Control guineapigs all reacted with febrile and scrotal reactions. Infection resulted in all three monkeys, but was mild. Fever was brief, and no dermal lesions occurred. Leucopenia developed in two, and a positive Weil-Felix reaction in all, agglutination being of the OX19 type of *Proteus* X strains.

DEMONSTRATION OF THE RICKETTSIAL NATURE OF THE RESPECTIVE VIRUSES.

Rural Typhus.—The rickettsia are present, often in large numbers, in guineapigs and rabbits infected with the virus; in the former, in the ascitic fluid and fibrin covering the spleen; and in the latter, when infected by the intra-ocular route, in the cells of the endothelium covering Descemet's membrane of the reacting eye. In smears made of such infected material, and stained by Giemsa's method, they are seen to lie within the cytoplasm of the cells, the majority grouped as one or more clusters near to the nucleus. Rarely, extra-cellular forms are seen. In morphology they are short rod-shaped bacilli that show bi-polar staining. The polar granules are coccoid in shape and purple in tinge; between them lies a connecting narrow rod that stains pale blue. Dimensions may be given as 0.8 to 2.0 μ in length and 0.3 to 0.5 μ in width. In morphology, distribution, and staining characteristics, they are identical with the *Rickettsia orientalis* shown by NAGAYO *et al.* to be the causal organism of the tsutsugamushi disease of Japan. (The authors are greatly indebted to Professor NAGAYO for the gift of smears of *R. orientalis* and *R. prowazeki*.)

Confirmation of the rickettsial nature of the virus of rural typhus was afforded by the histological examination by one of us (LEWTHWAITE, 1936) of brain material from seven patients dead of the disease. In five of them rickettsia were demonstrated. They were found in close relation to the nuclei of endothelial cells in lesions of the capillaries and small arterioles. Their

characteristics did not differ from those described above in experimental rural typhus.

The Tsutsugamushi Disease.—From the reacting eye of a rabbit infected by the intra-ocular route smears, made and stained precisely as described above in experimental rural typhus, reveal rickettsia that in numbers, morphology, distribution, and staining characteristics are identical with those of rural typhus.

Urban Typhus.—The rickettsia are found within the cytoplasm of the endothelial cells of the exudate covering the tunica vaginalis and parietalis of guineapigs that react with scrotal swelling. They are present also, though fewer, in smears from the reacting eye of a rabbit infected by the intra-ocular route, and in the fibrin covering the spleen of infected guineapigs. In some general characteristics they resemble *R. orientalis*. Thus they are bi-polar, show differential staining and a distribution as clusters, and extra-cellular forms are occasionally seen. But they are longer and thinner than *R. orientalis*, and the two polar elements are more linear; moreover, they are much fewer in number. Comparison with *R. prowazeki*, on the other hand, showed that these rickettsia of urban typhus did not differ in morphology, distribution, and staining characteristics.

THE RELATIONSHIP OF THE THREE DISEASES TO EACH OTHER AND TO ROCKY MOUNTAIN SPOTTED FEVER.*

Cross-immunity experiments were carried out in the guineapig, rabbit, and monkey; the criteria relied on were the clinical signs of infection and the Weil-Felix reactions.

Immunity to Re-infection with Homologous Virus.

Five strains of rural typhus were compared with one another: complete cross-immunity existed.

Two strains of the tsutsugamushi disease were compared with one another: complete cross-immunity existed.

Immunity to Re-infection with Heterologous Virus.

Strains of rural typhus and the tsutsugamushi disease have been compared. Complete cross-immunity exists. It may be emphasized here, as recorded earlier, that in experimental infections of monkeys with either virus by the intra-dermal route identical dermal lesions occur.

* Since these findings and those that follow comprise the substance of a series of detailed publications that are in the course of preparation, detail is omitted from this text.

Between strains of rural typhus and the tsutsugamushi disease on the one hand, and a strain of urban typhus on the other hand, there is entire absence of cross-immunity.

Dr. R. R. PARKER, Director of the Rocky Mountain Spotted Fever Laboratory, Montana, U.S.A., very kindly furnished to the authors a strain of spotted fever for study. Received in ticks, it was established in guineapigs with some difficulty; and studied in them and in rabbits for many months.

Comparison of strains of the rural typhus-tsutsugamushi group of diseases on the one hand and Rocky Mountain spotted fever on the other hand, shows an entire absence of cross-immunity between them.

Comparison of the urban typhus and the spotted fever strains shows that, when the second inoculum is the virus of spotted fever, there is entire absence of cross-immunity. When, however, the second inoculum is the virus of urban typhus, there is a very definite, though incomplete, cross-immunity; it may well be that this partial immunity is more apparent than real, and due to the differing virulence of the two strains, the spotted fever strain being very virulent for guineapigs and rabbits, the urban typhus strain much less so.

Our experience with Rocky Mountain spotted fever vaccine accords with the above results and suggestion. A batch of this tick-tissue vaccine, which is known to be efficacious against spotted fever and the typhus of Sao Paulo, was received from Dr. PARKER for trial in the experimental forms of the Malayan typhus fevers. It proved to have no protective value against them.

SEARCH FOR THE CARRIERS OF THE THREE TYPHUS-LIKE DISEASES.

This investigation was made possible by the collaboration of Mr. E. HODGKIN, Entomologist to the Institute for Medical Research, F.M.S.

It is certain that the body louse plays no part in the transmission of the Malayan typhus fevers; and it is thought, on the available epidemiological evidence, that larval mites will prove to be the carriers of the rural typhus and tsutsugamushi group of fevers.

The present search was restricted to the rôle of two species of ticks and one species of flea as possible carriers of rural and urban typhus.

RÔLE OF THE RAT-FLEA (*Xenopsylla cheopsis*).

Urban Typhus.—It has been demonstrated conclusively, in each of the series of experiments, that under laboratory conditions the rat-flea (*X. cheopsis*) can acquire a virulent infection with the virus of urban typhus from infected white rats by feeding, and that on transference to an uninfected white rat it transmits the infection to this rat.

Rural Typhus.—In the case of a parallel series of experiments carried out *pari passu* with the above series, in which the virus used was the Secrangayee strain of rural typhus, no evidence was forthcoming that the rat flea could acquire and transmit the virus under laboratory conditions. [Since the completion of the above investigation, one of us (SAVOOR) has repeated this attempt by a modified method, and has obtained evidence that may be interpreted as indicating that the rat flea has a feeble power to transmit the virus of rural typhus ; possibly the rat flea may prove to be a carrier of secondary importance.]

RÔLE OF TICKS.

Two species of ticks were used, one the American wood tick, *Dermacentor andersoni*, the other the dog tick, *Rhipicephalus sanguineus*. *D. andersoni*, which so far as is known is not found in Malaya, is the most important carrier of Rocky Mountain spotted fever, and was used at the suggestion of R. R. PARKER in the hope that it might prove, under laboratory conditions, a carrier of one or other of the typhus disease of Malaya, and so enable such virus to be sent to America for comparison with the many typhus strains there available. *R. sanguineus* is the carrier of fièvre boutonneuse and African tick typhus.

No evidence could be obtained that either species of tick could transmit or even acquire the virus of rural or urban typhus, although the procedure and technique did not differ in essentials from those known to be successful in demonstrating transmission of Rocky Mountain spotted fever.

EVIDENCE AS TO THE RESERVOIR OF THE MALAYAN TYPHUS FEVERS.

It was known from the work of FLETCHER and his colleagues, of GATER, and of ANIGSTEIN, that the probable reservoir of rural typhus was the rat. The authors have obtained additional evidence on this point.

Firstly, as noted earlier in this paper, they were able to maintain the virus in the white rat for twenty-one generations without any loss of virulence ; and so to demonstrate, by the criterion of NICOLLE, that rural typhus is a murine strain.

Secondly, they have recovered two strains of tropical typhus from two of twenty-three wild rats trapped in areas in which human cases of disease had occurred. Both strains were established in guineapigs. Amongst other signs of infection were scrotal swelling and abundant intra-cellular rickettsia in the tunica vaginalis. In rabbits inoculated, by either the intra-ocular or the intra-peritoneal route, with infected material from passage guineapigs, agglutination of the OXK type of *Proteus* X strains to titres as high as 1/1700 and 1/1925 have been obtained. A concomitant infection with *Spirillum morsus muris* was found in both of the rat strains ; it proved fatal ultimately to the guineapig, so that attempts to carry out cross-immunity experiments in guineapigs were abandoned. Use of rabbits, however, was more successful. One of these rat strains was established in rabbits by intra-ocular injection of infected material

from a passage guineapig, and maintained by the intra-ocular method of passage without difficulty. The other rat strain defied repeated attempts to establish it thus. Cross-immunity results were as follows. Rabbits convalescent after infection with a strain of the tsutsugamushi disease or any of four strains of rural typhus were immune to the virus of the rat strain; and conversely. In the case of a fifth strain of rural typhus, transferred from guineapigs to rabbits for the purpose, three convalescent rabbits of the rat strain showed complete immunity to re-infection with this strain, and three others a partial immunity.

ATTEMPTED VACCINATION OF GUINEAPIGS AGAINST EXPERIMENTAL INFECTION WITH RURAL TYPHUS.

The Russian workers, TZEKHNOWITZER and PALANT (1933), have reported the successful vaccination of guineapigs against infection with the virus of typhus exanthematicus. They obtained their success by using vaccines that consisted of either formalinized or carbolised brain-tissue virus. Two attempts have been made by the authors to achieve a similar successful vaccination by similar methods. The Seerangayec strain of rural typhus was used. In both, a total of 42 guineapigs were vaccinated at intervals with graded doses of a formalinized emulsion of brain-tissue virus, that varied from the dosage successful in the hands of the Russian workers to a dosage that was two-and-a-half times that given to a man in SEMPLE's method of anti-rabic vaccination at the Institute for Medical Research (assuming the dosage required for the guineapig to be one-thirtieth that required by man).

All attempts failed.

In conclusion, mention is made of five other infections that were met with in guineapigs in the course of the above investigations, infections which may resemble experimental typhus, and some of which may supplant the typhus virus. They were melioidosis, rat-bite fever, infections with a toxoplasma, with *B. enteritidis* Gaertner and with *B. paratyphosus* B.

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DISCUSSION.

Dr. A. Felix : It is now eight years since Dr. FLETCHER read before this Society the first paper on the two serological types of tropical typhus, and I remember how puzzling his results appeared, to me and to others, at that time. But his results soon came to be fully confirmed and amplified, and the work he then presented had a most stimulating effect on the study of typhus-like diseases in other countries. As a result of this work the number of so-called unclassified fevers of the tropics, and outside the tropics, has been reduced very considerably, and the "Typhus Group of Fevers" has been firmly established and still continues to grow. Dr. LEWTHWAITE's work is a most valuable contribution to the knowledge of this group of viruses, because it reinforces the experimental basis on which the present conception of the "Typhus Group" rests.

Dr. LEWTHWAITE has referred to NICOLLE's criterion for the demonstration of the murine or non-murine origin of typhus viruses. I have some doubts as to the significance of that criterion. We may take an analogy from the guinea-pig, which is one of the most susceptible experimental animals. Its susceptibility to infection varies enormously with the different types of typhus virus, although none of these viruses is a natural pathogen of the guinea-pig. It is possible that the rat behaves in a similar manner, quite apart from the well-established fact that the rat is known as a carrier of one or several types of typhus virus.

With regard to the vaccination experiments, Dr. LEWTHWAITE has been using a formalised suspension of brain-tissue virus, and with this he has obtained negative results. It is known from earlier work with European epidemic typhus, that there is a marked difference in the immunising effects of vaccines containing killed tissue virus or killed louse virus. The latter is an efficient immunising agent while the former is not. The nature of the difference is not known, though the last experiments of WEIL, which led to his fatal infection with the contents of louse-guts, seemed to indicate that there was a qualitative difference. It is also known that the vaccine against Rocky Mountain spotted fever is prepared from tick-virus and it seems quite possible that in the typhus group as a whole vaccines containing killed virus from the arthropod vector will be found to be superior to those containing virus from mammalian tissues.

As to the interesting serological observations which Dr. LEWTHWAITE mentioned in connection with a variant or mutant of one of his virus strains, it is for me very tempting indeed, to discuss these at some length. However, I shall resist the temptation, since these immunological details would lead beyond the scope of the present discussion.

Dr. William Fletcher : Tropical typhus appeared in the Malay States in 1924. I went there at the beginning of 1903, and during my first twenty years

we did not see cases of this kind. Then suddenly the disease began to occur. Before 1924, I can remember only one case which may have been tropical typhus. It resembled typhoid fever, but after the patient had been ill for some time, the temperature came down with a run, and we thought the reason was that an abscess had burst. But there was no sign that any abscess had been present.

About one-third of the population of the Malay States is Chinese, one-third Tamil, and the remaining one-third native Malay. The Malays live in the country districts away from the towns, and do not come into the town hospitals very much. One sees few cases of tropical typhus among them, and I should like to ask Dr. LEWTHWAITE if he thinks it probable that typhus smoulders among the Malays, as yellow fever does among the natives of West Africa.

Lt.-Col. L. T. Poole : It may be of interest if I give a classification which Major J. S. K. BOYD has adopted in India. For the past few years considerable attention has been directed to the typhus group of fevers, and BOYD has recently subjected to critical analysis 110 cases of typhus which occurred in India in 1934. He has endeavoured to group these cases on the basis of their serological reactions with different strains of *B. proteus*—XK, X2 and X19. Further, an attempt has been made to adopt a grouping according to the clinical types of the disease which have shown some regional variation.

To simplify matters the classification adopted by BOYD is reproduced (p. 575). The table has been taken from page 362 of his communication on the subject which appeared in the *Journal of the Royal Army Medical Corps* of November and December, 1935 (Vol. lxx, pp. 289-367).

XK Group.—The cases that occur under this heading are definitely demarcated both on serological reactions—the titre of the serum is usually high to OXK—and in their clinical aspects.

Clinically the cases are moderately severe. The rash when present is characteristic. It is distributed on the trunk, persists for the matter of 7 days and when it disappears leaves no staining on the skin. Serologically, whilst OXK is agglutinated to a high titre, as a rule there is practically no co-agglutination demonstrable for OX2 or OX19. It is of interest to note that some 60 to 70 per cent. of healthy normal individuals in India show a titre of from 1 in 25 to 1 in 50, or rarely higher for OXK. Heterologous agglutinins for the enteric group, it is stated, are usually elaborated.

Cases of this type of the disease have occurred widely throughout India, and there appears to be no definite regional distribution. They occur with maximum incidence in August, September and the early part of October. This somewhat restricted seasonal incidence suggests that the vector is present during the terminal part of the monsoon, and that it dies off during the drier and colder months. The vector is at present unknown, and no primary lesion has been detected in any of the cases in this group. Clinically and serologically

this type of the disease corresponds with the Malayan " rural " type of typhus.

X2 Group.—Up to the present, distinction between various types of typhus fever has been based upon the varying serum reactions to XK and X19, but so far, no type of the disease has been described in which X2 is the main antigen. BOYD, owing to demonstrable agglutinins being present for this organism in fourteen of his cases, has suggested a new group. In his series of cases, however, whilst OX2 was agglutinated to moderately high titre, OXK and OX19 also exhibited agglutination. It is evident from the serum reaction of these cases that this is a group response and that the strain of *Proteus* representative of the main antigenic component has not yet been isolated. Similarly to the previous group heterologous agglutinins for the enteric group are also common.

It is interesting to note that these fourteen cases appeared to have a definite regional distribution. They occurred at irregular intervals from September to January with an apparent maximum incidence in December.

On clinical grounds, whilst the cases were mild or moderately severe, the rash presented some points of difference from that noted in the XK group. In the latter group it has been mentioned that it was restricted to the trunk. In the cases under consideration it was much more generalised, petechial in nature, appearing both on the trunk and the extremities, and persisting for a very much longer period, leaving after its disappearance a brown staining of the skin.

As in the case of the XK group, the vector is not known, and there is no primary lesion. The histories of these cases associate them with camp and jungle life.

X19 Group.—The cases under this heading have been grouped mainly on clinical grounds, but even adopting this classification there appears to be more than one variety of the disease in the group, and so, an attempt has been made to sub-divide the group according to districts in which the cases conform to similar clinical types. The group has been divided into two sub-groups.

(i) *Poona-Ahmednagar sub-group.*—In clinical manifestations and seasonal incidence the cases of this sub-group closely resemble the X2 group, but on the whole are of a more severe nature. In their serological reactions whilst OX19 is agglutinated, the titres are low with marked co-agglutination for OX2 and OXK, in some cases the co-agglutination titres being almost as high as OX19 itself—obviously a group response. Heterologous agglutinins for the enteric group are freely produced in this variety of the disease, and are much more marked than in any of the other groups.

As is the case in the other groups the nature of the vector is hypothetical and there is no primary lesion. BOYD notes that in one case of the series it was possible that the patient had been bitten by a tick, but no definite evidence was available.

(ii) *Bangalore sub-group.*—Clinically, cases placed under this heading exhibit a mild course. The rash when present is comparable to that of measles,

TYPHUS FEVERS IN INDIA.

A SUGGESTED CLASSIFICATION (BOYD).

	XK	X2	X19	
			Poona-Ahmednagar	Bangalore
Geographical Distribution	Northern, Eastern and Southern Commands except Poona-Ahmednagar Area and Madras District. Not reported from Western Command	Deccan District and Poona Independent Brigade Area only	Deccan District C.P., and Poona Independent Brigade Area only	Southern Command except Poona Independent Brigade Area and Ahmednagar vicinity
Seasonal Incidence...	Maximum, August and September	Maximum, December	Maximum, December	More or less evenly spread except February, March and April
Rash—				
No. of cases * ...	Br.15/21, Ind.1/14	Br. 8/8, Ind. 5/6	Br. 10/10, Ind. 6/6	Br. 5/6, Ind. 1/21
Day of appearance	5th or 6th	Br. 3rd or 4th Ind. 3rd to 10th	Br. 3rd, Ind. (average) 7th	Br. 4th to 10th Ind. 8th
Type ...	Flush + macules	Macules, Papules, Petechial	Macules, Papules, Petechial	Maculo-papular
Distribution ...	Trunk only	Generalized	Generalized	4 cases trunk only, 2 cases trunk and limbs
Duration ...	7 days	Br. 18·4, Ind. 14·4	Br. 25, Ind. 10·5	Ind. 3, Br. average 4
Staining ...	Nil	Brown + in some cases	Brown + in some cases	
Duration of Pyrexia	Average 14·2 days	Average 12·5 days	15·5 days	10·4 days
Stay in hospital ...	Average 31 days	Average 27·5 days	Average 29·5 days	Average 24·6 days
Proteus agglutinins	K + + + 2 — 19 —	K ± 2 + + + 19 ±	K ± 2 ± 19 + to + +	K ± 2 ± 19 + + +

Br. = British ; Ind. = Indian.

* Numerator shows number of rashes, denominator shows number of cases.

appearing on trunk and limbs and persisting for about 3 or 4 days with no very definite seasonal incidence.

Serologically OX19 is agglutinated to high titre whilst OXK is equally agglutinated to low titre. This type both serologically and clinically resembles endemic "flea-borne" typhus. The vector has not been determined.

In India much work still remains to be done on this group of fevers. It presents a profitable line of research, and it is hoped that the attention which has been directed to them will result in their more correct appreciation.

It must be emphasised that the classification which BOYD has suggested is only a provisional one and that further research may require its amendment.

Lt.-Col. H. E. Shortt : I did not expect to speak to-night so late, but on the little I have seen of tick typhus in India must be from memory. It may be of interest to give these briefly, as a kind of comparison with the work Dr. LEWTHWAITE has been doing.

The cases I have seen of typhus in India have nearly all been in the plains areas, and in that connection there is some interest in what Colonel LEWTHWAITE has just been telling us with regard to the distribution of the cases and the year in which they occurred. I recently made a collection of the records of the recorded cases in India, and they, clearly, fell into two groups, those occurring in the cold weather, and those met with in the hot weather. The cases occurring in the cold weather are all cases in the Plains. In the hot weather the cases were contracted in the Hills, and it looked as if the vectors in either case were insects which liked conditions neither too hot nor too cold.

As to cases in Kasauli and hills round about, it seemed to be a local thing there. I have known that district for many years, and we used not to get many cases. But in the last 6 or 7 years the number of cases seen has been increasing. Working on this basis, I wondered if there was any new condition introduced, or previously existing. There has recently been introduced there the guinea pig from Madras. It was originally brought as an experimental animal, but they escaped, and later they overran the locality. They started at the end of the station, and they have worked towards the south. Most of the cases of typhus occurring locally have been in this northern area. We collected squirrels and examined their sera by the Weil-Felix reaction and we found that about 60 per cent. reacted with OXK emulsions in a low titre, not more than 25 or 50, but in some cases the titre went up to 250, and in one case up to 500. I collected a similar number of the same squirrels from the hills, and the picture I got in them was a very different one; instead of 60 or 70 per cent. reacting, as in the former case, only about 20 per cent. reacted, and with OXK. In neither group was there an appreciable reaction with OX19.

The results of animal inoculations from human cases were very

I remember, gave the most definite febrile reactions were rats and guineapigs. None of the animals showed anything in the way of marked serotal reactions. In a few of the guineapigs and a few of the mice, on examining the tunica there was seen to be a definite inflammation, and in some cases rickettsia bodies were seen, though in very small numbers, and in order to find them they needed to be carefully searched for.

Colonel COVELL has been working in the same area during the last year, and he has isolated a strain of virus from wild rats, and another strain from fleas. I think the emulsion he made in the latter case was from the flea *Ceratophyllus* sp.

With regard to initial lesions, we did not see them. Colonel COVELL contracted the disease himself, not as a result of the work he did on the subject, but he took up the work because he had been a victim of the disease. He had a small area on his flank, which showed a series of small lesions, closely packed together, looking like a series of mosquito or other bites, such as flea bites, and they remained throughout his long illness, and the area I speak of was still present two months later.

As to Dr. LEWTHWAITE's work, the impression I have derived is that the more work that is done on this series of strains of typhus, the more is the tendency noticeable to bring them closer together. Dr. LEWTHWAITE has told us that when he tested his rural typhus he could produce, by intra-dermal inoculation, local lesions as in tsutsgamushi disease; then he got a perfect cross-immunity between the two, which was not, however, repeated in the American strain. And, in the second place, he had a monkey which changed its serological reaction completely. This tends to show that we are dealing with organisms which are very closely related to one another.

There are a few questions which I would like to ask Dr. LEWTHWAITE. He has mentioned one or two instances in which he gave the titre shown by the animals. I wondered what the average titre might be, whether it remained high or low. The animals I spoke of went to a maximum of 500 in the naturally caught animals. I ask whether he made any attempts with his fleas to infect animals by means of the oral route. I also ask whether he made any attempts to obtain cultures of the virus by some of the methods now used such as in the chorio-allantoic membrane of chicks, or tissue cultures.

Dr. Lépine (of the Pasteur Institute, Paris): Dr. FELIX has raised, better than I could have done myself, the different points on which I was prepared to speak. There is one additional remark I wish to make: namely, that in French Indo-China the situation is almost the same as in the Malay States with regard to typhus fevers. One finds, as in the Malay States, different types of diseases belonging to the typhus group, which are often mistaken one for the other, though they can be distinguished either by their clinical course or by experimental findings, or by serological reactions.

First, in Indo-China we see typhus exanthematicus, the classical louse-borne disease. It was prevalent at a certain period, but is rare now. Still one can see some cases in mining districts of Annam, where they are introduced, usually, by Chinese coolies. There is nothing further to say about this type, except that the rash is very difficult to see in coloured people, and so we cannot be quite certain as to its absence which has been occasionally described as a particular characteristic of typhus fever in Indo-China. This type produces the Weil-Felix reaction in the usual manner, giving a high titre with *Proteus* X19.

Secondly, we see there, too, a milder form of typhus, which we call murine, following NICOLLE, and which corresponds to the urban type of typhus described in the Malay States. It is the same disease and produces the same symptoms in the guineapig: fever with scrotal swelling, but no fatality in the animals. The serum of patients agglutinates OX19 and often OX2 strains of *Proteus*, at a medium or low titre, but is inactive with the Kingsbury strain.

Dr. LEWTHWAITE referred to the difficulty of securing infected blood from patients suffering from urban typhus. This is true, as the blood is usually infective during the first 5 days, when the diagnosis has not, as a rule, been made because the patient has a negative Weil-Felix reaction. But there is now available a simple method of obtaining infected blood at any stage of the disease. We know that if the blood of the patient does not prove infective after the 5th day, it is not because there is no virus in the blood, but simply because, after that period, antibodies appear in the blood. The concentration of antibodies gradually increases and they become sufficient to protect the animal against the virus. For instance, if you take 5 c.c. of blood at any time during the course of the disease, as GIROUD has recently shown, and permit it to clot, then discard the supernatant serum, wash the clot with saline solution, grind it and inject the suspended clot intraperitoneally into guineapigs, one may recover the virus long after the first days of the disease. And this holds true also for the disease in guineapigs. By the same method, virus can be recovered from guineapigs inoculated with murine typhus or typhus exanthematicus, even after the febrile period has ceased, occasionally 21 days after inoculation. This endemic typhus is rat-borne and is transmitted by fleas. All fleas are apt to be infected by the virus. In fact, in 95 per cent. of the cases, it is *Xenopsylla cheopis* which is responsible for transmitting the virus from rat to rat and occasionally from rat to man. In some cases there may be infection by the oral route in human beings, especially in instances where patients have been ingesting food contaminated by the urine of rats, which in itself may be virulent.

A third disease which is encountered in Indo-China belongs to the group of tsutsugamushi disease of Japan. I agree with Dr. LEWTHWAITE when he says that tsutsugamushi and rural typhus are the same disease differing only in the presence or absence of an ulcer. In Mediterranean countries there is a good example of a disease of a similar type, although much milder. It is the fièvre boutonneuse, transmitted by the dog tick. As Dr. LEWTHWAITE told you, it is

true that the "tache noire," which may be compared to the ulcer of tsutsugamushi fever, may be present or absent: it is always the same disease, namely, fièvre boutonneuse. The presence or the absence of the "tache noire" or ulcer may be due to the quantity of virus deposited at the site of the inoculation.

In any case, both from clinical descriptions and from experimental studies, the disease met with in Indo-China is obviously very close to and probably identical with tsutsugamushi and rural typhus. This fever gives a positive Weil-Felix reaction with OXK strains as in the Malay States and Japan. But an interesting point is that in Indo-China we do not yet know which vector is responsible for transmitting it. Tsutsugamushi in Japan is transmitted by *Trombicula akamushi*, whereas in the Malay States, it is transmitted by *Trombicula deliensis*. Is it one of these mites or both, or is it a third one which transmits the same virus in Indo-China? We do not know; the fact is that Indo-China being geographically placed between China and Japan on one side, and the Malay States and the Dutch Indies on the other side, provides a link between the Far Eastern and the Indonesian diseases.

Maj.-Gen. Sir John Megaw: I had intended to attack Dr. LEWTHWAITE in connection with the following statement which appears in the proof copy of his paper—"In this paper the tsutsugamushi disease and rural typhus are referred to as separate entities in virtue of the clinical distinction met with in human cases, *viz.*, the occurrence of an initial ulcer and bubo in the former, and their absence in the latter"; but in his spoken word he has forestalled my criticism by telling us that the initial ulcer and bubo might not be enough to differentiate between the diseases; he then went on in his paper to show, as clearly as anyone could show, that there were no grounds for differentiating between these two. I can add another piece of evidence on this point: I have seen in a definite case of tick typhus in India a local ulcer and bubo; the ulcer was at the site where the tick was found, so that there could be no doubt about the diagnosis although in the great majority of cases of tick typhus in India no local ulcer and no bubo is found. What Dr. LEWTHWAITE himself has said is sufficient to show that the local lesions are more or less fortuitous and not matters of essential importance.

The only other point on which I disagree with Dr. LEWTHWAITE is, that he still sticks to a local classification of the disease. He may, perhaps, claim to be in good company in that respect, because in other countries people insist on their own particular classification; often their patriotism is such that they give the name of their country to the disease; or, if they do not do that, they employ some other equally unsuitable name.

I think Dr. LEWTHWAITE's work has helped very much to clear up the subject; he has made it plain that in Malaya there are two types of the disease, one which is conveyed by fleas, and the other by mites. In India we have abundant evidence of the existence of the kind which is conveyed by ticks; it

between types of typhus fevers. In Malaya most cases of the tsutsugamushi disease have been very severe whether in young or old. Yet I can recall four cases at least, two of them in elderly debilitated men, characteristic in every sign and symptom, that ran a very mild course.

With regard to the development of my theme that rural typhus and the tsutsugamushi disease should no longer be regarded as separate entities: it was necessary at the outset to postulate a difference between them—the usually accepted clinical one—in order to compare and contrast the results of our experiments on the viruses derived from patients of these clinical types, and to show thereby their identity.

COMMUNICATIONS.

RURAL HYPER-ENDEMIC MALARIA IN TANGANYIKA TERRITORY.*

BY

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From the Malaria Unit, Tanga, Tanganyika Territory.

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I.—THE RATIONALE OF THE PRESENT INVESTIGATION.

One of the most salient features that present themselves to an observer of anti-malarial effort during the past few years is the frequency of errors arising from the application of knowledge acquired in one part of the world to the solution of problems occurring in another. In spite of the vast amount of

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The work here recorded owes a large share of any merit it may possess to the work of my wife, MARGARET E. WILSON, M.B., CH.B.

malaria survey and investigation, which has been steadily increasing in volume and intensity since the discovery of the cyclical transmission of malaria in 1897, anti-malarial measures are still largely empirical. The emphasis which has been laid on mosquito control has led to neglect of the other aspects of the malaria problem, and advances in the parasitology of malaria have also been hindered by the crudeness of clinical practice in the tropics; in fact more progress has been made in this direction in the few years that have elapsed since the initiation of malaria therapy for the treatment of general paresis, and the opportunity this gave for precise and controlled observation, than in the previous three decades. With regard to the human reaction to infections, while the obvious differences in the reactions of tolerant and non-tolerant persons, or groups of population, were too obvious to be missed, there is little or no information available as to the physiological or sociological reasons for these differences.

It follows from this that anti-malarial measures cannot be applied from a general knowledge of the natural laws of malaria, but only from a precise knowledge of the conditions actually found in the locality in question.

The present study is an attempt to acquire more exact information concerning the incidence and course of malarial infection in an African (Bantu) population living in a hyper-endemic area; to determine the events which underlie the commonly accepted indices of malarial intensity, namely the spleen rate and the parasite rate, to establish in fact a standard by which other communities may be judged; and also to estimate the degree of disability likely to ensue from such infection. This information is, we believe, a primary need for any anti-malarial measures that may be attempted in tropical Africa whether for pure native or for mixed communities. In the case of the latter the source and reservoir of infection is always native malaria, and it is difficult, if not impossible, to understand truly the more complex conditions of malaria in mixed communities until they are elucidated by the study of the more simple events occurring in untreated homogeneous populations.

Apart, however from its relevance to the malaria of mixed communities (such as the many small towns and the industrial or agricultural undertakings under alien enterprise) such study has an importance of its own. For the bulk of the population of Central Africa is, and will remain, a rural population. A large proportion of this rural population lives in areas where malaria is hyper-endemic, and while it is commonly assumed that under such conditions there must be much morbidity and a corresponding economic loss, there does not appear to be adequate careful observation to substantiate this conclusion.

Thought about malaria under these conditions appears to be too much dominated by the findings in epidemic malaria, such as occurs in Northern India, and in mixed or immigrant communities, such as estate labour; in either of which both the circumstances and the problem are very diverse from those of African rural communities.

II.—SUMMARY OF PREVIOUS LITERATURE.

Surprisingly few observations have been made on pure native communities living in hyper-endemic areas, and while there are a number of reports on the results of blood examinations of children (and occasionally adults) in different parts of tropical Africa, they are all inadequate in their ecological data, and rarely refer to more than a single set of examinations.

So far as I have been able to ascertain, all the more complete African studies refer to semi-urban or urban and, therefore, mixed communities.

SCHÜFFNER (1919) has given the most complete discussion of hyper-endemic malaria, based on his studies in Sumatra. He pointed out that infections decrease in a typical manner with age (benign tertian before quartan, and quartan before subtertian, infections), and explains this sequence, not by the decreasing number of relapses, but by the trend of all attacks to run to a natural cure, while the length of time required for a cure decreases as immunity is acquired. This is supported by the rapid decrease in the gametocyte rate (from 51 per cent. in children under 2 to 8 per cent. in adults). The relationship found between the spleen (92 per cent.) and parasite rates (37 to 38 per cent. in children, and 8 to 11 per cent. in adults) is contrary to the results of workers in other tropical hyper-endemic areas (see CHRISTOPHERS *infra*); but HELFFERICH (1934) has reported the presence of a high parasite rate with a lower spleen rate, also in Sumatra. SCHÜFFNER also found that while the death rate was about 10 per cent. higher than in non-endemic areas in Sumatra, the birth rate was the same.

In a more recent contribution SCHÜFFNER, SWELLENGREBEL, ANNECKE, and DE MEILLON (1932) have compared these findings with those in Bantu in South Africa. They find that in the case of the Bantu the parasite rate reaches a peak of 100 per cent. in children of "play age," and never falls below 40 per cent.; while the spleen rate in adults is nearly 50 per cent. as compared with nearly 100 per cent. in children. They explain the difference as being due to fundamentally diverse types of reactivity to malarial infection. In the Malay the reaction is to suppress completely the parasites of each fresh infection, and there is a persistent splenic enlargement corresponding to this activity. The Bantu, on the other hand, has an inherited tolerance to malarial infection which results in a kind of commensalism, a tolerance which is reflected in the lesser degree of splenic enlargement and the fewer enlarged spleens.

CHRISTOPHERS (1924) studied a community in the mining villages of Singhbhum in Bengal by the more refined method of parasite counts. He found that the parasite rate rose to 100 per cent. in the third year of life and remained high throughout childhood, while the average parasite count dropped from 10,659 per c.mm. in the second year to 7,124 in the third year, and then again to about 1,000 per c.mm. for the rest of childhood. The spleen rate rose to 92 per cent. between the ages of 3 and 5, and then fell to 74 per cent. In adults there were few parasites, little signs of malaria, and the spleen rate dropped to 11 per cent. He concluded that in a high state of endemy a substantial degree of immunity was acquired during the first two or three years of life, with the result that while "attack" conditions were present continuously for the first two years of life ("acute infestation"), they were only present once in 6 months in adults ("immune infestation"). Gametocyte rates were highest in the second year, and were associated with acute infections and a lack of immunity. He failed to establish any correlation between malarial infection and anaemia, and noted that these children appeared to be in a fair state of health after the first two years.

The picture presented by CHRISTOPHERS is questioned by KLIGLER and MER (1933), who state that infection in children in Palestine does not begin to decline until after the fourth year. KLIGLER and REITLER (1928) had previously recorded the occurrence in Palestine of seasonal epidemics in their "hyper-endemic" area. But though the mean spleen rate was in the region of 75 per cent. the mean parasite rate in children under 4 was only 35.2 per cent., rising to 45.4 per cent. in the age group 5 to 9. In one group of villages the parasite rate in children under 4 rose from 20.9 per cent. to 48.3 per cent. during the epidemic season, and from 7.2 per cent. to 26.5 per cent. in adults. There was thus a greater seasonal increase in adults than in children, and the conditions in the area studied were obviously very different from those in Singhbhum. These authors

vidently failed to appreciate the great difference in liability to infection in Palestine, where malaria transmission is seasonal, as compared with hyper-endemic areas in the tropics, where transmission is continuously or almost continuously present. A similar contrast is drawn by REICHENOW (1929) between Hausas, in West Africa, and Bulgarians.

PERRY (1913) remarked on the lack of apparent effect of malaria on the physique, at any rate of adults, of the aboriginal tribes in the Jeypore Hill Tracts of the Madras Presidency. These people had a parasite rate of 80 per cent. and a spleen rate of 80 to 90 per cent.

Both he and PHILLIPS (1924) compare the aborigines with more recent Aryan immigrants, and find a great difference in the effectiveness of their respective responses to malaria, the latter having much the greater susceptibility even after years of settlement in a hyper-endemic area. They have a higher spleen and lower parasite rate as compared with the aboriginal tribes (compare SCHÜFFNER *ante*).

It is therefore evident that there is a good deal of discrepancy between the findings in different races and under differing conditions of hyper-endemicity. While it is difficult to believe that the fundamental human reaction to malaria can be different in different races, there is not yet enough evidence to bring these conflicting findings into coherence. It is, however, apparent that the events which are found to occur in one race cannot be explained in terms of the findings in another.

Turning to Africa, the records are found to be less complete. In one of the earliest studies, STEPHENS and CHRISTOPHERS (1900) give a general description of native malaria in Nigeria and the Gold Coast. They state that there is a condition of infection in native children without febrile disturbance, and a condition of active immunity in the adult accompanied by a progressively scantier development of parasites. DANIELS (1901) also stated that fever was common in native children, but rare in adults. The spleen rate rose to a maximum at the third year and then declined.

The earlier figures given for the parasite rate are comparatively low, owing to the use of thin films, as for example ANNETT, DUTTON, and ELLIOTT (1901) and SOREL (1911); and more modern authors often fail to state whether thick or thin films were examined. Many authors have recorded series of examinations which show the decrease of parasitisation in children with advancing age, among them LEGER (1914) in Nigeria, LEGER and BAURY (1922) in Senegal, BARRETO (1927) in Portuguese Guinea, SCHWETZ and BAUMANN (1929) in the Congo.

REICHENOW (1929), working among Hausa children, found a steady decrease in the number infected after the age of 5; before this 100 per cent. were infected, and in the age-group 16 to 20 only 55 per cent. He thought it an exaggeration to say that malaria was an illness of black children.

In Sierra Leone, MACDONALD (1926) showed that in an area where the average parasite rate in children was 41 per cent., and the spleen rate 50 per cent., there was no reduction up to the age of 12 years; in another area where the parasite rate and spleen rate were both 72 per cent., definite reduction occurred after the age of 10 years. Both these areas were urban, and not hyper-endemic to the extent that is commonly found in rural areas in East Africa. Another mixed urban population in the vicinity of Lagos was studied by BARBER and OLINGER (1931) who found that the parasite rate rose from 28 per cent. in the first year to 98 per cent. at three years and then fell to 76 per cent. between 13 and 15; over 16 it was only 48 per cent. The number of heavier infestations began to fall earlier, namely, after 2 years of age, when it was 78 per cent. of all infections, to a level of only 9 per cent. after the age of 16. From the results of observations on African employees, they conclude that adults never acquire a complete tolerance to malaria; but they come to no conclusion as to the importance of malaria in causing infantile illness.

VAN NITSSEN (1933) found that a maximum parasite rate of 96 per cent. occurred between the ages of 2 and 3 in the Congo, but this was accompanied by little illness.

THOMSON (1934) examined 103 Nyasa village children monthly, over a period of a year (by thin films). Only four of these children were consistently negative, but there were no very severe infections. He found no decrease in the frequency of *Plasmodium falciparum* infections, but both *P. vivax* and *P. malariae* had disappeared by the age of 10. He concluded that the development of tolerance was a gradual process but that its development was unaccompanied by signs of illness.

Another village community was studied by GARNHAM (1935) in Kenya. He reports that over a period of one year there was no apparent seasonal change in the parasite rate, that symptoms were mild, and that each adult had on an average only one attack of fever annually. All children were infected within 6 months, *P. falciparum* and *P. vivax* preceding *P. malariae*. The parasite rate declined during childhood to a level of 25 per cent. in adults. Out of four infant deaths which occurred, one was attributable to malaria.

These and other papers by observers in Africa concur in the conclusion that malaria has its efflorescence in childhood, as shown by the high but decreasing parasite rate at that age. They also give the impression that probably in childhood, but certainly in adult life, malaria causes comparatively little illness in Africans. None of them, however, throw much light on the nature or origin of immunity except by inference, and there are some discrepancies which are difficult to explain.

III.—THE ENVIRONMENT AND THE PEOPLE.

1. *The Locality.*

The villages which are the subject of this investigation lie about 15 miles to the North West of Tanga, which is on the East African coast in the North of Tanganyika. The three villages are referred to collectively as Gombero, a much larger village in their near vicinity. They were chosen on the grounds of malariousness and stability of population; they lie away from any main routes, and are believed to have as stable a population as any in the vicinity of Tanga; they are not liable to extraneous influences, such as European estates and missions, which might affect their malariousness.

Mgandi and Mwengere, the villages concerned, lie about 1 mile on either side of the Msimbazi, a seasonal river 15 to 30 feet wide during the rains, but drying up to a chain of pools during the dry season.

During the rains water also collects in a number of depressions, and the pools so formed persist for a period after the rains, one of them only drying up towards the end of the dry season. There are a few shallow wells, some used for drinking and others for bathing.

Two-thirds of the houses are built of mud, one-third of plaited coconut leaf, both types are roofed with a thatch of coconut leaf. About equal numbers are one, two or three-roomed; none have any windows; one in six has a separate hut for cooking; and a quarter have very insanitary pit latrines. The average number of persons to a house is about 3.5.

Nearly everyone sleeps on a bed, the children sleeping with their mother; there are two mosquito nets in all.

2. *Climate.*

There was a wide departure from the normal seasonal variation during the period of this survey. The mean rainfall distribution at Tanga for the 12 years, 1921-32, was as follows:—

TABLE I.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Mean	2.0	2.4	4.7	9.4	8.5	3.4	2.3	2.6	3.1	3.8	4.5	3.1	49.8
1933	5.6	5.1	3.8	4.4	3.7	1.1	5.2	3.7	1.5	1.5	4.6	1.4	41.6

There is, then, a main rainy season from March to the beginning of June, followed by a long dry period, and a shorter "rains" in October or November. The cycle was interrupted (see Table 1) from October, 1932, until the early part of 1934, although there was a number of heavy isolated downpours. This failure of three rainy periods caused a serious food shortage at Mwengere, and a lesser shortage at Mgandi.

The direct effect on the observations here recorded was to make the year 1933 unrepresentative of any seasonal changes which occur in this area.

There are comparatively small variations on the coast in the temperature maxima and minima during the year; so small (from 28° to 32° C.), that they can play no important part in the ecology of anopheles.

No observations have been made on humidity, but the climate at Gombero is slightly drier than that of the coast proper. The rainfall for the 12 months, December, 1933, to November, 1934, was 42 inches, of which 9 inches fell on 4 days in March and 17 inches in June and July. The total for Tanga was practically the same for the period, but the mean temperatures at Gombero are rather higher and humidity is also reduced by distance from the sea.

3. *The People.*

With the exception of three Wanyamwezi, the population of these villages consists entirely of members of the Digo tribe, a Bantu tribe of northern origin which has lived at or near the coast for several generations at least.

The total number examined at the several two-monthly examinations has been 443—233 at Mgandi and 210 at Mwengere; but at any one time not more than 360 are actually resident in these villages. Some of the difference is accounted for by births (28) and deaths (11), some by marriage changes (this affects children as well as adults, for young children remain with the mother), some by men, with or without their wives, going out to work on plantations, and the remainder mostly by visits to relations. Forty men are known to have gone away to work for periods up to a year or even more, and 170 other persons are known to have been away for some days or longer, while many more have probably not been recorded. Twenty have left, apparently not to return.

It will be observed that the hoped-for stability has not altogether been realised. On the other hand, hardly any strangers come to these villages, and the movements which do occur are, in the great majority of cases, to and from places within 10 to 15 miles, in which the malarial status is very similar.

The age composition of the villages is as follows:—

TABLE II.
POPULATION IN AGE GROUPS.

		0-5	6-10	11-20	Over 20			0-5	6-10	11-20	Over 20
Mgandi	M	19	18	19	58	Gombero	M	50	35	23	99
	F	9	13	19	78		F	37	23	46	94
Mwengere	M	30	16	19	46	Vunde	M	48	35	7	70
	F	18	12	5	64		F	43	7	21	65
Total		76	59	62	246	Total		178	100	99	328

The enumerations at Gombero itself and at Vunde (4 miles east of Gombero) were made as a check on the findings for the selected villages, and there is sufficient correspondence to give a certain amount of confidence that the group examined is typical of this area.

4. *Food and Economic Resources.*

The staple food is cassava, together with various kinds of small beans ; maize, sweet potatoes and millet are also eaten to a small extent. Fat is obtained from coconut and various kinds of green leaves are an adjunct to the main food. During their season many mangoes are eaten. Owing to the drought of 1933, the diet at that time was so reduced (more particularly at Mwengere) that obvious malnutrition resulted, particularly in children. The drought was later accompanied by locusts, which proved a most valuable addition to the diet.

Although 1933 was exceptional, there is nearly always a quantitative food deficiency at the end of the dry season. Qualitative deficiencies are present all the time in varying degree. These are a shortage of fats and of vitamins, the deficiency of the latter being greatest towards the end of the dry season, when both greens and beans are scarce. The main vitamin deficiency is in the A factor.

Infants are breast-fed up to a year or even longer, but from the age of a few weeks breast-feeding is supplemented by starchy foods of a varying degree of unsuitability.

Little improvement of diet or of the rest of the physical environment could be maintained without a rise in the economic level, which has declined greatly in the last few years. At one time copra (dried coconut) was a considerable source of wealth, now the price has dropped and it is hardly worth preparing. Cattle were another source of wealth, but these are decreasing owing to East Coast fever, the advance of trypanosomiasis and other diseases. There are still a few cattle whose milk is sold. Meat is a coveted luxury, and the amount of it consumed would form a good index of the general economic level. Goats are a form of capital, only drawn upon in emergency.

In paid employment on sisal estates it takes these men 6 weeks or more to complete a card of 30 days' tasks, as they are quite incapable of working continuously for more than a few days at a time ; for this they receive 12 to 15 shillings.

Regarded physically, the final as well as the first impression of the inhabitants of Gombero is their utter inability to make an adequate response to any stimulus ; their quietness, their slowness and their poor physique. The complement to this is their deteriorating economic status, which their physical condition provides no reserves to meet.

5. *General Morbidity and Mortality.*

The necessity for not demanding too much of the people being examined unfortunately prevented that detailed survey for the presence of other diseases which would have completed the background for the specifically malarial observations of this survey. Numbers of the people did, however, present themselves, or their children, at our dispensary for treatment. The chief complaints in the age-group 1 to 5, were of "chest" (in which case a capillary bronchitis was often present), of "fever" (which was confined to this age-group), and of "stomach" (which we believe to be generally a symptom of malaria), diarrhoea was a less common but distinct complaint. A purulent gingivitis was almost universally present at this age, but only occasionally complained of. In the age-group 6 to 20, the chief complaints were of yaws and schistosomiasis. In adults, the commonest complaint was of constipation, closely followed by "chest" which included two cases of chronic bronchitis, one of pleurisy and one of pneumonia, but was most commonly a manifestation of some sort of cardiac dysfunction (tachycardia, etc.) due to ankylostomiasis. Yaws and gonorrhoea were both common in adults.

Tuberculosis.—There have been two deaths from this disease, and we have also made a clinical diagnosis of pulmonary tuberculosis in two other cases without being able to confirm it on a single examination of the sputum. It seems probable that more careful examination of the whole population would have revealed further cases.

Ankylostomiasis.—Stool examinations have been made of the whole population at Mgandi, excluding children under 1 year. The results are shown on p. 588 :—

TABLE III.

INCIDENCE OF ANKYLOSTOMIASIS AT GOMBERO.

Age.	Negative.	Positive.		
		1	2	3
0—5	5	8	3	1
6—10		7	8	6
11—20	1	19	7	2
21—30		10	13	2
31—40	1	11	9	5
41—50		8	7	5
Over 50		6	9	1

Number examined, 153 : Ankylostome ova present in 146. Ascaris ova in 3. Trichostrongylus ova in 2, together with ankylostome ova in both cases.

Degrees of infection (after concentration by salt floatation) : 1.=Less than 1 to a field (magnification $\times 80$) ; 2.=1 or 2 to a field ; 3.=Several or many to a field.

It may be seen from Table III that, after the age of 5 (more correctly after the age of 3) ankylostomiasis is present in 99 per cent., and that the heavier infections are increasingly present after that age. It cannot be adduced as an important cause of anaemia earlier than this. For discussion of the relationship of anaemia and ankylostomiasis, see pp. 613, 614.

Schistosomiasis.—Soon after they begin to go to the pool to bathe, children begin to be infected, that is about the age of 6. From 7 or 8 up to the age of 20 boys are universally infected, and while practically all are passing some blood all the time, half of them are passing it in fairly considerable quantity, and in a few the urine is brown and thick with blood and other cells. The heaviest infections are in the first half of the period. While some girls are infected, a greater number are not : the number examined is not great enough for more precision than this. In adult life, the number of males with active infections steadily decreases with advancing age, and the severity of the haematuria in a corresponding degree. The proportion of women who have been infected increases somewhat in adult life. The period at which schistosomiasis is likely to be an important factor in the causation of anaemia is then between the ages of about 7 and 25, or less.

Causes of Death.

There have been 11 deaths in a population of about 420 during a period of 20 months, a death rate of 15.7 per 1,000. This may be compared with the results of an investigation which is being conducted, largely on members of the same tribe, in the coastal area of Kenya (PHILIP, 1934), but on a population of over 50,000. There the death rate is 20.19. The infantile mortality rate in this area was 125, compared with 148.5 in Kenya.

The details of the eleven deaths were as follows :—

- (1) m. 62 years. Strangulated hernia.
- (2) f. 37 " . Salpingitis and peritonitis.
- (3) m. 27 " . Haemoptysis.
- (4) m. 12 " . Disseminated tuberculosis, peritonitis.
- (5) m. 1½ " . Diarrhoea (not seen).
- (6) m. 1 year . " Chest " (last seen 2 weeks before); died 3 months after first infection with malaria, very heavy infection and severe anaemia, also rickets.
- (7) m. 9 months. " Chest " (last seen 3 weeks before); no complaint of " fever " and malarial infection had fallen to under 3,000 per c.mm. Death not attributable to malaria except as an important contributory cause.
- (8) m. 3 months. No history (last seen 3 weeks before death). Had malarial infection of over 20,000 per c.mm. Death mainly attributable to malaria.
- (9) m. 2 months. Never seen. History of " stomach " but not " fever." (But see p. 589.)
- (10) m. 5 weeks. Malaria. Film taken 3 days before death showed an infection of over 20,000 per c.mm. and one segmenting subtertian parasite.
- (11) f. 2 weeks. Prematurity (never seen).

PHILIP ascribes 11·8 per 1,000 of infant deaths to malaria.

6. Birth Rate and Survival.

The birth rate, of 40 per 1,000, corresponds fairly closely to the figure of the Kenya investigation, which was 49·36.

As an attempt to relate these births, over the short period of less than 2 years, to the more permanent status of the population, the children born to each woman are here compared with similar figures from the Union of South Africa (1932).

TABLE IV.
BIRTHS AND SURVIVAL.

	Europeans South Africa.	Africans Urban Port Elizabeth.	Africans Rural South Africa.	Gombero.
Number investigated... ..	Census Total 1926	309	460	114
Average age	37	39·9	—	35·5
Average number of births per woman	3·59	6·06*	8·65	2·75
Average number of children died ...	0·62	2·99	4·33	1·17
Average number of survivors ...	2·97	3·07	4·32	1·58
Deaths per 1,000 births, in lifetime of mother	173·5	493·6	500·6	426·7

* Includes premature and still births.

In preparing the figures for Gombero (Table IV), all women above the age of 18 were included, this being the approximate age of marriage. On the assumption that 45 may be taken as the upper limit of the child-bearing age, 32 women whose child-bearing was complete had 36 children surviving or an average of 1.13.

While allowing that these numbers are very small, and no more than an index of what is happening in the areas round about, they do at least suggest that the level of fertility is very low indeed. The general impression gained from our experiences of these villages is that the low survival is due to infantile rather than child mortality. Survival is, however, appreciably greater than in the South African native groups.

7. Medical Treatment.

The only medical treatment available in the area was provided by an African dispenser, who was one of the staff of this survey. With the exception of injections of bismuth sodium tartrate for yaws and syphilis, he gave no specific treatment of any kind for any disease to inhabitants of the selected villages. The people themselves were indifferent to treatment of any kind unless their disease were very painful, and would take no trouble to obtain it. They make spasmodic use of various barks and leaves for the treatment of fevers and other diseases, but the use of such medicaments, supposing that they possess any activity, was too infrequent to play any important part in the reduction of malaria.

IV.—THE VECTOR, ITS PREVALENCE AND INFECTIVITY.

1. Breeding Places.

With one exception, all collections of larvae have been taken in two types of water, either in pools in the river Msimbazi, or in the rain water pools that have their origin during the rains. Even during the rains odd collections of larvae are found in river pools, but more often in the dry weather before the river has dried up completely; the river is, however, the less important source of anopheles, so far as these villages are concerned. During and after the rains, each of the rain pools carries many thousands of larvae, *A. gambiae* vastly predominating.

A. funestus (type) is comparatively uncommon, breeding amongst reeds at or near the edges of both types of pool, but mainly in the largest and most permanent one. There is at this pool both a greater depth of water and a deeper canopy of reeds.

A. coustani (*mauritanus*) (type) also selects the deeper shade on both types of pool. It is not very common.

A. squamosus is found mainly in the rain pools at the edges, but in the slighter shade only.

2. House Infestation.

Only *A. gambiae* and *A. funestus* have been caught in houses, the proportion of the former to the latter being 20 to 1 (See Table V). The following observations cover a period of 16 months, from August, 1933, to November, 1934, and are based on 2,087 house searches, each house being gone over completely once only.

The average number of anopheles per house over the whole period was 5.6, males 1.1 and females 4.5. Of the two types of house construction, mud houses, average 6.2, seemed to be more attractive to anopheles than those walled

with cocoanut leaf, average 5.0. The reasons suggested for this difference are that, owing to their more open structure, coconut-leaf houses offer less perfect shelter, they are also lighter and drier than mud-built ones. There is some difference between the infestation in Mgandi and Mwengere; at the former the mean of female anopheles per house is 4.1 and males 1.0; and at Mwengere females 5.2, males 1.2.

The seasonal variations in house infestation are shown in Table V and Figure 1 (pp. 594, 595). The variation in the number of female anopheles per house is from 0.6 to 14.9, the variation occurring during 1934 being probably fairly typical, while that of the latter part of 1933 is not, for the reason already explained on pp. 587, 588. There are, then, always quite appreciable numbers of anopheles, but it is only for three or four months of the year that they are present in large numbers; anopheline infestation depends on rainfall which is great enough to form accumulations in the pools and river bed.

Since June, 1934, a series of more complete searches has been made in parallel with the searches which provided the data given above. In each village six houses were chosen as catching stations; this eliminates variation due to differences in structure. Only three or four houses were searched in a morning, and each was gone over until no more mosquitoes could be found. The proportion which the results of the two methods show to one another is 98 to 45; in other words, twice as many mosquitoes can be caught by prolonged searching.

It is, therefore, evident that the true house infestation would be *at least* double that shown in Table V, and almost certainly even greater than this.

As approximately one-third of the houses were searched weekly and the effect of this, more especially in the dry season, would have been fairly considerable, all anopheles caught, with the exception of those retained for dissection, were released when the day's catching had been completed.

3. *Anopheline Infectivity.*

Infectivity has been measured by gland examinations only, except in a few of the earlier batches, when midgut dissections were also made. The general result of these examinations is to show a sporozoite rate of 12.2 per cent. (Mwengere 14.4 per cent. and Mgandi 11.1 per cent.).

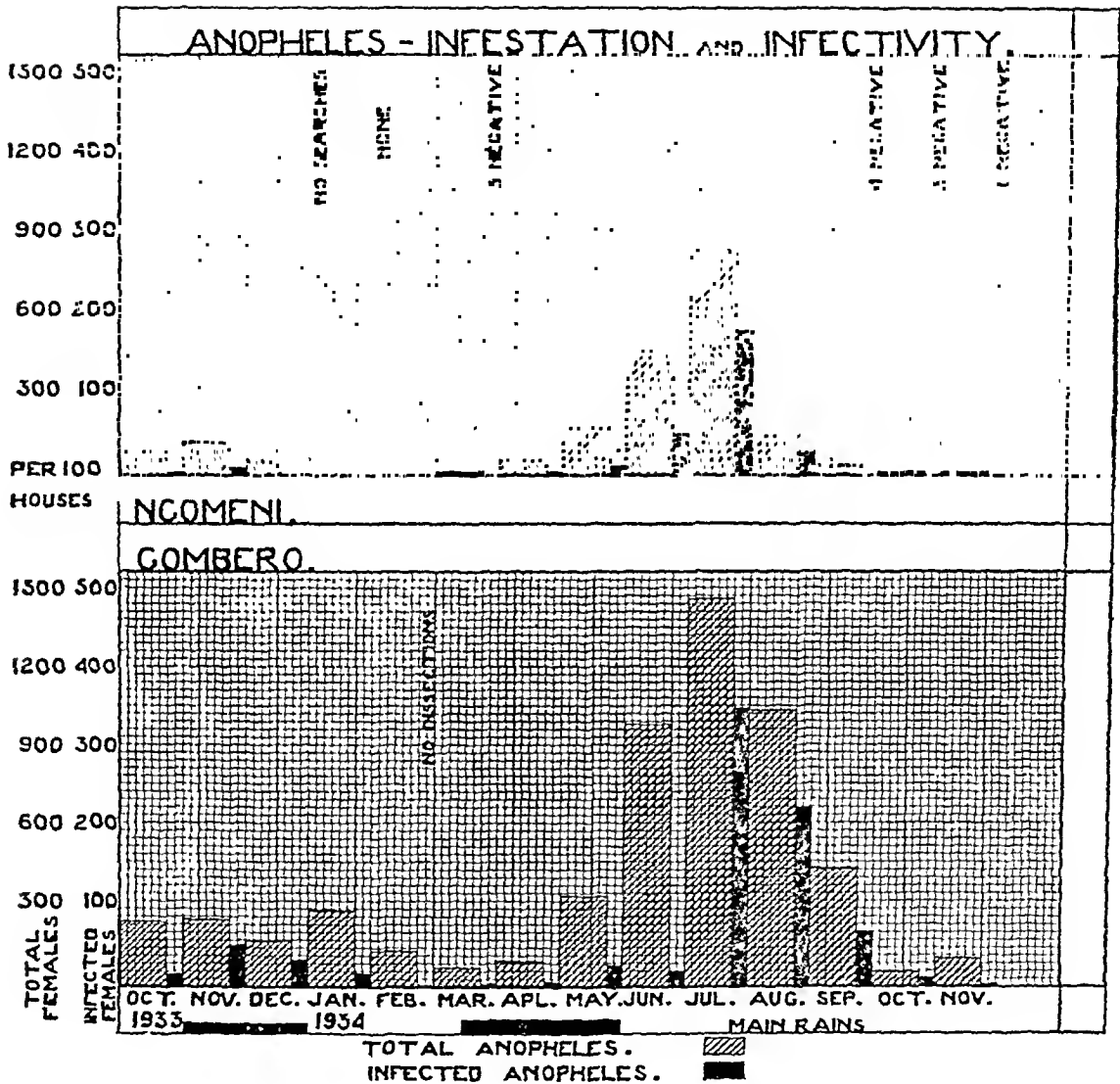
The seasonal variations which occur are shown in Table V and Figure 1 in which they are compared with those which occur at Ngomeni, south of Gombero, a place at which breeding is always more scanty. These histograms are substantially the same, the difference being those of degree only. They both show a rise in the numbers of anopheles and in the proportion of infected anopheles following very rapidly after the onset of the rains. Even a very transient replenishment of the rain pools causes a fair anopheline rise, as in October-November, 1933, and an increase in infectivity, as in November-December of the same year.

TABLE V.
SUMMARY OF MOSQUITO FINDINGS AT CONIBERO.

Date.	Houses Searched.	Anopheles Caught.					Glands Dissected.		Female Anopheles per House.	Sporozoite Rate.	Infected Anopheles per House.
		Male.	Female.	<i>A. gambiae.</i>	<i>A. funestus.</i>	Not Identified.	Pos.	Neg.			
1933											
Aug.	62	24	62	33	—	53	4	28	1.0	12.5	0.13
Sept.	63	61	92	16	—	137		16	1.5	0.0	0.0
Oct.	137	127	345	106	2	360	6	89	2.5	6.3	0.16
Nov.	100	110	263	83	—	290	17	66	2.6	20.3	0.53
Dec.	90	64	188	25	—	227	4	21	2.1	16.0	0.33
1934											
Jan.	75	46	222	66	—	202	4	66	3.0	5.7	0.17
Feb.	182	58	269	—	—	327			1.5	—	—
Mar.	180	53	276	140	—	189	4	136	0.8	2.9	0.02
Apr.	158	171	551	150	—	572	8	142	1.0	5.3	0.05
May	178	155	629	51	—	339	4	46	3.5	8.0	0.28
June	194	424	1,965	158	1	2,230	3	156	10.1	1.9	0.19
July	100	210	1,493	152	7	1,444	61	193	14.9	24.0	3.58
Aug.	181	448	1,938	99	22	2,265	26	95	10.7	21.5	2.29
Sept.	185	264	840	76	17	1,011	14	77	4.5	15.4	0.70
Oct.	65	16	39	46	9	—	16	70	0.6	18.6	0.11
Nov.	137	58	150	34	—	171	1	33	1.1	2.9	0.03
									Means		
Totals	2,087	2,280	9,322	1,235	58	9,820	172	1,234	4.5	12.2	0.55

Midgut dissections 236; Oöcyst rate 9.7 per cent.

FIGURE 1.



This rise in anopheline infectivity is not due to a precedent rise in human infectivity, on the contrary the latter follows rather than precedes it. It appears that there must be some biological difference in the parasite-vector complex consequent on rain, which causes or permits a more rapid and more certain development of sporozoites.

The maximum infectivity of 24.0 per cent. in July, 1934, is the highest that has been recorded in this survey, and corresponds to a known house infestation of 3.58 infected, and probably infective, anopheles per house and to a probable infestation of at least 7 per house. Assuming that a female anopheles feeds every fourth night and that there are 3.5 persons per house, each person

sleeping in one of these houses during this month of highest infestation was likely to receive an infecting bite every other night. At the end of the dry weather on the other hand, the chance of an infecting bite was only 1 in 300 on any one night.

It must not, however, be assumed that infection occurs only *inside* the house; it may occur outside as well. We have observed anopheles biting out of doors, and also on most of the few occasions that we have continued work in these villages in the open after dark one of us has been infected. (When we remained in our camp, which was sufficiently far from the village to have very few anopheles, we were never infected in a much larger number of visits.)

The essence of the anopheline situation in these villages seems to be that transmission is due to *A. gambiae*, which is dependent on rain for its breeding places and for acquiring its maximum infectivity; that the locus of infection is in or about the house; and that whatever measures of personal protection were adaptable to these scantily-clad people would be quite inadequate to protect them from frequent infection and re-infection for at least three to four months in the year.

V.—THE COURSE OF INDIVIDUAL INFECTIONS.

1. *Methods*.—Thick and thin films have been taken from all persons examined, at two-monthly intervals. These films were air dried, the thin films stained by the Shute modification of the Leishman technique, and the thick by either Geimsa stain or Kleine's eosin-azur II method.*

All blood films have been examined and counted by either my wife (Dr. MARGARET WILSON) or myself; the sole purpose for which African assistance has been used being to search thin films for parasites, which, when found, were seen by one of us.

Thick films have been used for counting parasites and, in a small proportion where there was no reasonable doubt, for diagnosis of species. In the majority (about 90 per cent.) the thin film was used for diagnosis. Parasites have been counted against leucocytes in preference to Sinton's fowl cell method for a variety of reasons. It is extremely difficult to maintain an even distribution of fowl cells, and as in addition parasites are not uniformly distributed in thick films, it appears that this method can only be an approximate and comparative one. While counting against leucocytes is probably an even greater approximation, it is one which is universally applicable without special equipment or practice. The clinching consideration for the purpose of this survey was that a varying number of slides were taken by Africans, and others, who could not be expected to learn the method. The leucocyte standard is based on a series of 80 white cell counts of persons of all ages at Gombero.

*Staining for one hour with a mixture of 0.1 c.c. of 1 per cent. eosin and 1 c.c. of 0.16 per cent. azur II, in 20 c.c. of distilled water.

It is proposed to give in this section an account of malarial infection from birth to adult life, as seen in this community, mainly from the clinical point of view. The figures on which this account is based will be given in Section VI.

2. *In Infancy*.—Owing to the length of the interval between the examinations and the reluctance of parents to allow examination of quite new-born children, a precise statement of the time of occurrence of the first infection of the children at Gombero is not possible. It can, however, be stated that all cases examined within the first month (except in the period immediately after the rains) were negative. The general condition of the babies at this early period of their lives was excellent. They were fat and placid, usually peacefully asleep, and generally in very much better health than the mothers who had borne them.

A month or two later the same child, who had previously been so healthy would present a very different picture. To the most casual observer the child was evidently ill; it was fretful and feverish, snuffling and constipated. On examination at or very soon after the first finding of parasites, the haemoglobin had dropped from about 85 per cent. to about 45 per cent., the spleen had enlarged up to or beyond the umbilicus, high temperatures were recorded and a congestive condition of the lungs, which is difficult to classify, was often present. This state persisted for 6 months or rather more, and then, except for the few cases which ended fatally, improvement began to be seen.

It cannot be pretended that this striking change is solely due to malaria. The grossly unsuitable diet that begins to supplement breast feeding a few weeks after birth is at least an important concurrent factor in its causation. But the sequence of parasitization, together with the rapid drop in haemoglobin, point to malaria as its prime cause.

Twenty-five babies have been observed from birth for a varying period up to 19 months, and of these, six typical records are given in Table 6. The critical age period is in all cases to some extent conditioned by the date of birth in relation to season, but the appearance of the first infection was never later than 5 months after birth, while the average was 2 months.

This first infection was usually with *P. falciparum* alone, but if sometimes accompanied by *P. malariae* or *P. vivax*, these were only seen in comparatively small numbers. The usual sequence was for *P. malariae* to be added to the blood picture after an interval of 2 to 4 months, and *P. vivax* or *P. ovale* even later.*

During the period of acute infestation, which lasts for the first 18 months after the first infection, very high parasite counts are found,† oftener in some

*The presumed explanation of these findings is that the more virulent suppresses the more benign infection, an explanation which is supported by the work on monkey malaria by SINTON and MULLIGAN, and others, who have shown that underlying an apparently pure virulent infection there were latent one or more milder species.

†Counts of over 20,000 per c.mm. were all included as that figure in order to save the great expenditure of time involved in very high counts. The result is that the actual average figures given for the earlier years of life are a good deal too low.

TABLE VI.
GOMBERG. BLOOD EXAMINATIONS IN TYPICAL INFANTS.

Age in Months.	Case 97.	Case 7.	Case 172.	Case 58.	Case 193.	Case 192.
0						S 80
1						
2	neg.	neg.	neg.		SB 16,200	
3			SQ	neg.		Sg 20,000
4	neg.	S	SQB 2,400		SQB 15,600	S 5,300
5				S 20,000		
6	S 3,200	SQ 1,200			SQB 3,400	S 20,000
7			SQgO 10,200	SQ 2,100		
8	S 240	S 11,000			SQ 9,300	SQgBg 5,300
9				SQ 6,000		
10					SQ 5,300	
11		SQ 1,520				
12	SQ	S 7,200	SgQOg 3,800	SQ 12,800		SQgBg 19,200
13				SQ 20,000		
14		800	SQgO 11,800		SQ 6,800	SQgBg 14,000
15	SQOg 2,320					
16				SgQg 19,900		
17	SQ 1,800	SQ 3,700	SgQO 16,500			
18				SgQgBg 20,000		
19		SQB 11,500	SgQgO 20,000			

S = *P. falciparum*. Q = *P. malariae*. B = *P. vivax*. O = *P. ovale*. g = gametocytes.

The figures represent the numbers of parasites per c.mm. (over 20,000 included as this figure).

than in others. But in most cases counts of over 20,000 per c.mm. were seen once or more, and more frequent examinations would probably have revealed them in some of the remainder. One of the striking features of this period is the difference in the degree of infestation in different individuals. These babies were constantly being re-infected by fresh invasions of sporozoites: the differences cannot, therefore, be due to variation in the parasites, but rather to a variation in individual resistance.

The average count for this first year was more than 7,800 per c.mm., and this degree of infestation showed little abatement during the second year, when it was more than 7,200 per c.mm. In spite of the persistent parasitization the degree of anaemia begins to diminish, and by the age of 2 the critical period is undoubtedly passed. The stage of critical illness attributable to malaria is in fact a good deal shorter than this, and it is probably justifiable to regard an infant as being out of direct danger from malaria 6 months after its first infection.

The second year is the period at which all three or four (including *P. ovale*), species of parasite are most likely to be found.

It is also the period during which the gametocytes of one or more species are almost certain to be present. It is, in fact, when the infection is past its zenith that infectivity becomes a characteristic of these children. Neither when they are immune, nor before they have acquired their immunity, but when they are in process of the change-over to the immune state is their most infective period. It is unfortunately impossible to present this conclusion statistically, as it depends too much on the general physical condition in relation to the parasite count.

Very soon after the first infection great enlargement (up to or beyond the umbilicus) of the spleen occurs, and there is no noticeable decrease during the first two years of life.

It is notable that two, and perhaps three, out of the total of six infant deaths were directly attributable to malaria. This is in accord with the severity of infection seen in the majority of babies.

3. *In Childhood*.—The third, fourth and fifth years of life are characterized by the instability of the malarial status. They might, in fact, be described as the period of *semi-immune infestation*. In all individuals the parasite infestation is swinging irregularly from high to low with corresponding but lesser upward swings; the counts rarely fall below 50 and equally rarely rise above 10,000 per c.mm. Differences in individual susceptibility are accordingly less obvious at this period.

Fever is irregularly but often present, pyrexia up to 100° F. being frequently recorded. The spleen begins to be relatively less palpable as tolerance is increasingly established, and the general condition is much improved. In all but one or two individuals, children of this age run about and play as if they had nothing the matter with them.

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2			SQ 6,300	neg.		Sg 20,000
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4						
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7	S 240	S 11,000			SQ 9,300	SQgBg 5,300
8						
9	S 40	SQ 1,520	SQ 1,900	SQ 6,000	SQ 5,300	
10						
11	SQ	S 7,200	SgQOg 3,800	SQ 12,800		SQgBg 19,200
12						
13						
14	SQOg 2,320	S 800	SQgO 11,800	SQ 20,000	SQ 6,800	SQgBg 14,000
15						
16						
17	SQ 1,800	SQ 3,700	SgQO 16,500	SgQg 19,900		
18						
19		SQB 11,500	SgQgO 20,000	SgQgBg 20,000		

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Fever is irregularly but often present, pyrexia up to 100° F. being frequently recorded. The spleen begins to be relatively less palpable as tolerance is increasingly established, and the general condition is much improved. In all but one or two individuals, children of this age run about and play as if they had nothing the matter with them.

After the age of five, malaria shows a constant decline, a decline that has a consistency which is absent in the three preceding years. This consistency is reflected in the parasite counts, which rarely rise above 1,500 per c.mm. Negative blood films are occasionally found, and become increasingly common towards the end of childhood. The decline in parasite infestation may, however, be interrupted to some extent by any great rise in the infected anopheline infestation, as occurred, for example, in the months of July and August, 1934.

After the fifth year *P. vivax* is very rarely seen, but *P. malariae* is still common and may be found alone, although *P. falciparum* remains the dominant infection in most cases. Symptoms are absent whatever the species of the infection.

Gametocytes become increasingly uncommon and are never present in the large numbers found during the second and third years of life.

TABLE VII.

GOMBERO. BLOOD EXAMINATIONS IN TYPICAL CHILDREN.

Date.	Case 79. Age 2½-4½ years.		Case 96. Age 2-4 years.		Case 165. Age 3-5 years.	
1933 June	SQ	18,000	S	136	S	3,980
Aug.	SQg	2,500	S	1,190	S	680
Nov.			S	260	SQB	4,170
1934 Jan.	Sg	540	SgQ	270	SgQ	2,110
Mar.	SQB	4,420	SQ	2,240	SQB	448
May	SQg	3,990	SQ	680	Sg	1,260
July	SgQB	1,120	Q	5	SgQBg	2,100
Oct.	SgQ	7,400	Q	50	SgQB	1,400
Dec.	SQ	18,000	SQ	300	SgB	920
	Case 28. Age 7-9 years.		Case 141. Age 7-9 years.		Case 158. Age 10-12 years.	
1933 June	Sg	140	+	30	neg.	
Aug.	S	2,030	Q	60	B	220
Nov.	SQB	780	S	30	S	60
1934 Jan.	S	5	neg.		S	120
Mar.	SgQ	4,000	Q	30	S	80
May	Sg	670	S	2,520	S	60
July	SQ	280	S	140	S	60
Oct.	SgQ	950	S	60	SQ	80
Dec.	SQB	1,620	S	350	S	220

S = *P. falciparum*; Q = *P. malariae*; B = *P. vivax*; g = gametocytes; + = parasites present, but species not identified. Counts given to the nearest 10 per c.mm.

4. *In Adolescence and Early Adult Life*.—At this age the degree of infestation has fallen to a level which it substantially maintains for the rest of life ; the stage of " *immune infestation* " has in fact been fully achieved. Completely parasite-free individuals are a rarity, but the finding of any great number of parasites is an equally great rarity. The parasite rate declines during this period from about 60 per cent. to 40 per cent. Young people, and more particularly young women between the ages of 17 and 20 years, possess that same ebullition of vitality which is seen at this age even in non-tropical countries and people. It appears, therefore, that whatever part malaria may play in lowering the physical condition of communities in hyper-endemic areas, that part has already been played before adolescence.

Some qualification must, however, be made in the case of those individuals who for one reason and another spend periods in other localities. These result in fresh infections, which may lead to a varying degree of temporary slight disability. These infections were not sufficiently great in number to produce an appreciable effect on the rates at Gombero, but they appeared to

TABLE VIII.
GOMBERO. EXAMINATIONS IN TYPICAL ADULTS.

Date.	Case 6. Age 20-22 years.	Case 154. Age 20-22 years.	Case 113. Age 32-34 years.
1933 June	neg.	S 40	neg.
Aug.	S 2,040	S 40	+ 50
Nov.	neg.	S 100	neg.
1934 Jan.	neg.	neg.	neg.
Mar.	+ 40	S 170	Q 70
May	S 20	neg.	neg.
July	S 10	neg.	neg.
Oct.	S 70	neg.	neg.
Dec.	neg.	S 60	S 5
	Case 169. Age 30-32 years.	Case 2. Age 36-38 years.	Case 46. Age 45-47 years.
1933 June	neg.	+ 20	neg.
Aug.	neg.	S 100	S 10
Nov.	SQ 2,760	S 240	neg.
1934 Jan.	S 50	Q 60	S 220
Mar.	S	S 70	S 70
May	S 40	+ 5	neg.
July	S 20	neg.	neg.
Oct.	neg.	neg.	neg.
Dec.	S 40	+ 5	S 120

S = *P. falciparum* ; Q = *P. malariae* ; B = *P. vivax* ; g = gametocytes ; + = parasites present, but species not identified. Counts given to nearest 10 per c.mm.

After the age of five, malaria shows a constant decline, a decline that has a consistency which is absent in the three preceding years. This consistency is reflected in the parasite counts, which rarely rise above 1,500 per c.mm. Negative blood films are occasionally found, and become increasingly common towards the end of childhood. The decline in parasite infestation may, however, be interrupted to some extent by any great rise in the infected anopheline infestation, as occurred, for example, in the months of July and August, 1934.

After the fifth year *P. vivax* is very rarely seen, but *P. malariae* is still common and may be found alone, although *P. falciparum* remains the dominant infection in most cases. Symptoms are absent whatever the species of the infection.

Gametocytes become increasingly uncommon and are never present in the large numbers found during the second and third years of life.

TABLE VII.

GOMBERO. BLOOD EXAMINATIONS IN TYPICAL CHILDREN.

Date.	Case 79. Age 2½-4½ years.	Case 96. Age 2-4 years.	Case 165. Age 3-5 years.
1933 June	SQ 18,000	S 136	S 3,980
Aug.	SQg 2,500	S 1,190	S 680
Nov.		S 260	SQB 4,170
1934 Jan.	Sg 540	SgQ 270	SgQ 2,110
Mar.	SQB 4,420	SQ 2,240	SQB 448
May	SQg 3,990	SQ 680	Sg 1,260
July	SgQB 1,120	Q 5	SgQBg 2,100
Oct.	SgQ 7,400	Q 50	SgQB 1,400
Dec.	SQ 18,000	SQ 300	SgB 920
	Case 28. Age 7-9 years.	Case 141. Age 7-9 years.	Case 158. Age 10-12 years.
1933 June	Sg 140	+ 30	neg.
Aug.	S 2,030	Q 60	B 220
Nov.	SQB 780	S 30	S 60
1934 Jan.	S 5	neg.	S 120
Mar.	SgQ 4,000	Q 30	S 80
May	Sg 670	S 2,520	S 60
July	SQ 280	S 140	S 60
Oct.	SgQ 950	S 60	SQ 80
Dec.	SQB 1,620	S 350	S 220

S = *P. falciparum*; Q = *P. malariae*; B = *P. vivax*; g = gametocytes; + = parasites present, but species not identified. Counts given to the nearest 10 per c.mm.

4. *In Adolescence and Early Adult Life.*—At this age the degree of infestation has fallen to a level which it substantially maintains for the rest of life; the stage of “*immune infestation*” has in fact been fully achieved. Completely parasite-free individuals are a rarity, but the finding of any great number of parasites is an equally great rarity. The parasite rate declines during this period from about 60 per cent. to 40 per cent. Young people, and more particularly young women between the ages of 17 and 20 years, possess that same ebullition of vitality which is seen at this age even in non-tropical countries and people. It appears, therefore, that whatever part malaria may play in lowering the physical condition of communities in hyper-endemic areas, that part has already been played before adolescence.

Some qualification must, however, be made in the case of those individuals who for one reason and another spend periods in other localities. These result in fresh infections, which may lead to a varying degree of temporary slight disability. These infections were not sufficiently great in number to produce an appreciable effect on the rates at Gomboro, but they appeared to

TABLE VIII.

GOMBERO. EXAMINATIONS IN TYPICAL ADULTS.

Date.	Case 6. Age 20-22 years.	Case 154. Age 20-22 years.	Case 113. Age 32-34 years.
1933 June	neg.	S 40	neg.
Aug.	S 2,040	S 40	+ 50
Nov.	neg.	S 100	neg.
1934 Jan.	neg.	neg.	neg.
Mar.	+ 40	S 170	Q 70
May	S 20	neg.	neg.
July	S 10	neg.	neg.
Oct.	S 70	neg.	neg.
Dec.	neg.	S 60	S 5
	Case 169. Age 30-32 years.	Case 2. Age 36-38 years.	Case 46. Age 45-47 years.
1933 June	neg.	+ 20	neg.
Aug.	neg.	S 100	S 10
Nov.	SQ 2,760	S 240	neg.
1934 Jan.	S 50	Q 60	S 220
Mar.	S	S 70	S 70
May	S 40	+ 5	neg.
July	S 20	neg.	neg.
Oct.	neg.	neg.	neg.
Dec.	S 40	+ 5	S 120

S = *P. falciparum*; Q = *P. malariae*; B = *P. vivax*; g = gametocytes; + = parasites present, but species not identified. Counts given to nearest 10 per c.mm.

do so in the case of the township of Tanga, and caused a more considerable effect on the labourers of a sisal estate at Ngomeni near Tanga.

5. *In Adults*.—The only interruptions to the even tenor of the immune state in adult life appear also to be due to visits to other areas. There is also a rise, very slight in Gombero but greater in Tanga, in the number of parasites present in the blood during the period following the rains. The total number of cases in which, as a result of both these causes, the parasite count rose above 1,000 per c.mm. was only 25. There were only two examinations at Gombero in which the count rose above 8,000 in adults. The variation in individual tolerance is apparent in the accompanying illustrative summaries of blood examinations. Of 246 adults examined 12 only were negative on six or more occasions.

VI.—MALARIA IN THE COMMUNITY.

1. *Introductory*.—Epidemic malaria in the full sense of the term is not, so far as I am aware, known in Tanganyika Territory, although the possibility of its occurrence in connection with the non-immune communities at present living in the malaria-free zones is not so remote as it might at first sight appear.

The malaria that is present in the villages studied is essentially hyper-endemic having, as described in the preceding section, the characters of a high degree of infestation during the early years of life, and a lesser infestation with freedom from symptoms after childhood. This is CHRISTOPHERS' conception of "acute infestation" and "immune infestation," which he first applied to communities very similar to those which we have studied.

During the whole period of investigation 3,292 blood films were examined, of which 61.05 per cent. were positive. There was a small difference between the rates at Mwengere, 63.75 per cent., and Mgandi, 58.57 per cent. These correspond with the differences in the anopheline infectivity at the two places.

2. *Variations in Parasite Incidence with Age*.—Excluding the short initial delay before the first infection, 100 per cent. of children under four years are infected, and there occurs a corresponding maximum parasite infestation* of 7,875 per c.mm. during the first year of life. Following this first efflorescence, although the parasite rate still remains at 100 per cent., there is a rapid decline in the parasite infestation till it reaches its minimum level at about 14 or 15 years of age, the mean level from this time on being 177 per c.mm. The parasite rate, however, declines more slowly, and does not reach its minimal level until well on into adult life, falling to 67.9 per cent. at age 10, and to a mean of 45.3 per cent. during adult life.

The full results are shown in Fig. 2 (p. 603) and Table IX (p. 604). They correspond extremely closely to the results of CHRISTOPHERS (1924) and are in general agreement with the various previous findings in hyper-endemic areas in tropical Africa.

*This term is used hereafter for the average parasite count among infected persons.

FIGURE 2.

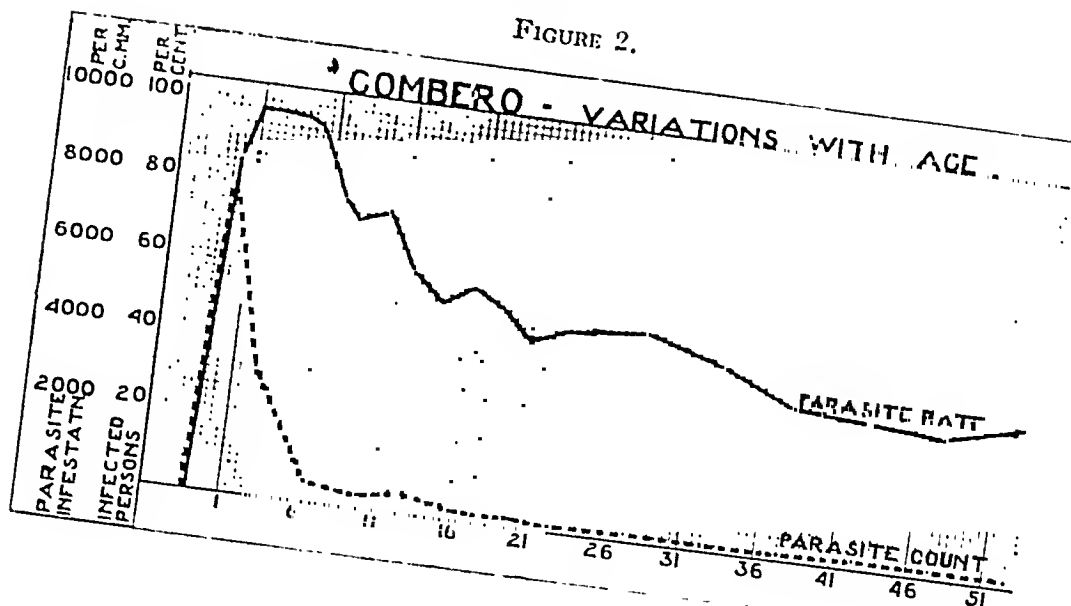
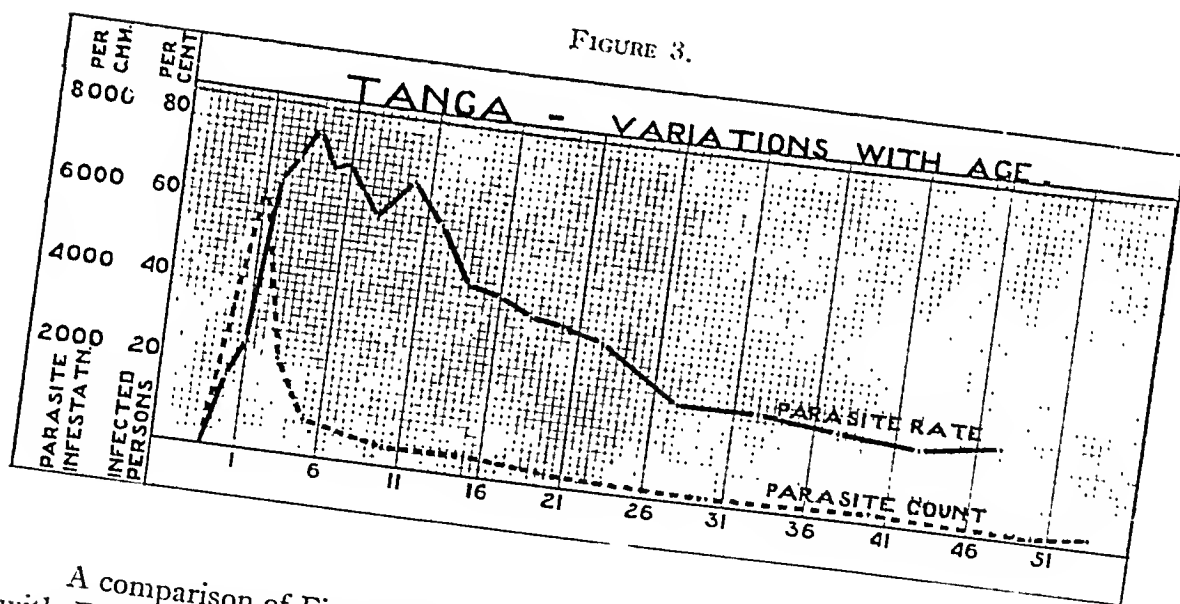


FIGURE 3.



A comparison of Fig. 3 (which is based on the same methods of observation) with Fig. 2 shows the difference made by a lesser degree of endemicity and a greater degree of instability of population on this curve. The mean parasite rate at the small coastal town of Tanga was only 44.7 per cent. (compared with 61 per cent. at Gombero), so that the level of parasitization is less at all ages; in addition to this, there is a slower rise and fall of both the parasite rate and parasite infestation. Such a comparison is of some value in the correlation of the results of workers on rural and urban malaria.

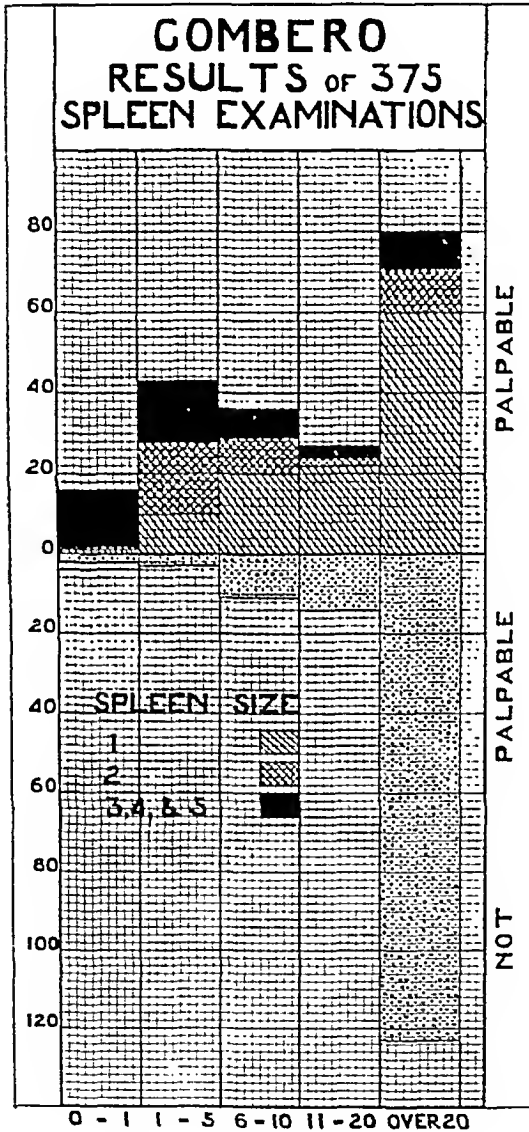
TABLE IX.

GOMBERO. AGE VARIATION IN PARASITE RATE AND INFESTATION

Age.	Total Examined.	Positive.	Per cent.	Infestation Index.
0-1	120	105	87.5	7,875
1	75	75	100.0	7,176
2	94	94	100.0	4,732
3	100	100	100.0	2,702
4	86	84	97.7	1,796
5	79	77	97.4	828
6	94	83	88.3	466
7	81	64	79.0	791
8	81	60	74.1	427
9	86	72	83.7	325
10	56	38	67.9	450
12	82	51	62.2	646
14	52	29	55.8	174
16	103	62	60.2	236
18	66	37	56.1	203
20	137	66	48.2	245
21-25	314	164	52.2	169
26-30	250	136	54.4	202
31-35	206	99	47.6	178
36-40	270	107	39.6	180
41-45	150	57	38.0	226
46-50	156	57	36.5	209
Over 50	288	120	41.7	172

3. *The Spleen Rate.*—Spleens were examined in the standing position by SCHÜFFNER's method, and the size numbers given in Fig. 4 are those obtained by this method.

FIGURE 4.



Attention has already been drawn to the rapid enlargement of the spleen which follows the first infection, and it is found that there is a preponderance of these large spleens in infants. This is succeeded by a progressive decrease in the size, and in the number, of enlarged spleens, during childhood and adolescence. The spleen rate (age 2 to 10) is 85 per cent., and the adult spleen rate 39 per cent. The fall with age is as follows:—

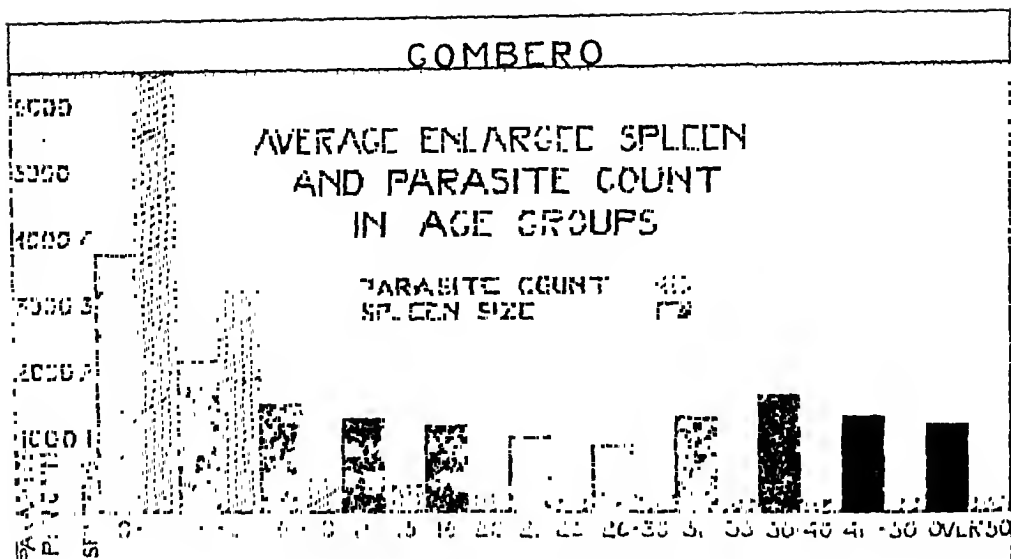
Under 1	..	89 per cent.
2 to 5	..	93 „
6 to 10	..	77 „
11 to 20	..	44 „
Over 20	..	39 „

It is evidently of great importance that the samples of children taken in the determination of the spleen rate should be correctly distributed among the different ages.

A secondary rise in the average enlargement occurs during adult life, as is shown in Fig. 5, which may be due to the summation of the effects of repeated re-infections, or, on the other hand, to the added effect of other diseases such as ankylostomiasis.

The results shown in Figs. 4 and 5 lend support to the theory of the spleen rate elaborated by CHRISTOPHERS (1924, 1927) and more particularly that the greater degrees of enlargement represent the result of a continuous state of infection; but that they also connote immunity seems to be less certain. We have observed that individuals with a great degree of enlargement tend to

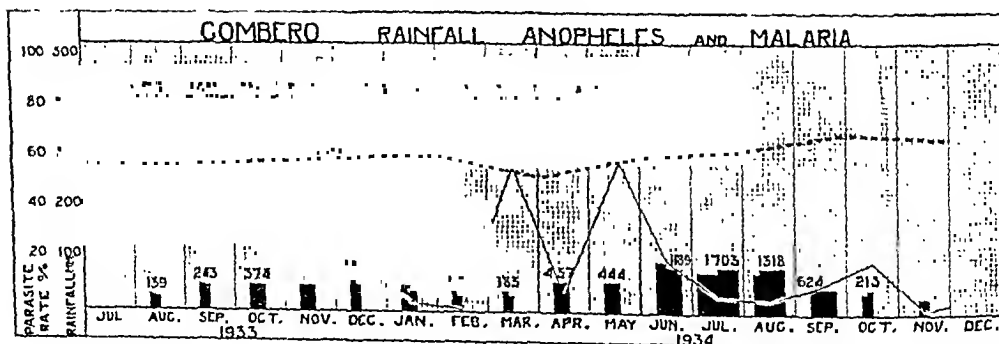
FIGURE 5.



master their infections more rapidly, but it still remains true that the increasing immunity of childhood is accompanied by a diminishing spleen, it may even be an impalpable one. Immunity must, in the light of other work already quoted, depend on activity of the spleen, as a part of the reticulo-endothelial system, but the presence of a palpable spleen is evidently not a corollary of its lesser activity in the fully-developed immune state.

4. *Seasonal Variation in Parasite Incidence.*—The seasonal variations in the parasite rate are shown, together with rainfall and the results of house catches of anopheles, in Fig. 6. The results of blood examinations are also summarised in Table X (p. 607).

FIGURE 6.



During and following the long rains of 1934 there was a small rise in the parasite rate, but the most noticeable feature of this rise was its insignificance. The lack of a corresponding rise in 1933 was due to the shortage of rain and

TABLE X.

GOMBERO. SUMMARY OF BLOOD EXAMINATIONS.

Month.	0-1		1-5		6-10		11-20		Over 20		Total Examined.	Positive.	Per cent. Positive.
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.			
1933													
Apr.	2	0	38	0	29	16	20	31	72	101	309	161	52.1
June	9	2	47	1	31	11	25	21	81	105	333	193	58.0
Aug.	12	2	41	2	30	9	31	23	76	104	330	190	57.5
Oct.	16	2	43	1	32	10	33	17	75	97	326	199	61.0
1934													
Jan.	9	2	40	0	34	9	29	15	91	98	327	203	62.1
Mar.	10	6	47	0	35	11	20	32	75	113	349	187	53.6
May	17	1	47	0	38	9	26	82	82	100	341	210	61.6
July	18	0	45	1	44	2	28	20	83	97	338	218	64.4
Oct.	11	0	40	0	44	2	33	14	105	80	329	233	70.8
Dec.	9	1	42	0	43	3	34	9	87	81	309	215	69.6
Totals	113	16	430	5	360	82	279	203	827	976	3,291	2,009	61.0

consequently of anopheles; while the rise in 1934 would presumably have been even less apparent but for the long preceding dry period. The general upward trend of the parasite rate is partly (perhaps 1 to 2 per cent.) attributable to improved staining of the films.

This slight seasonal rise in the parasite rate is due in part to more rapid infection of babies after birth, and for the rest to a fairly evenly distributed increase among all ages over five.

TABLE XI.

INFESTATION FIGURES AT TWO-MONTHLY EXAMINATIONS.

1933			1934			1934		
July	..	705	January	..	776	July	..	1,071
September	..	690	March	..	790	September	..	1,069
December	..	725	May	..	935	December	..	889

(These figures represent the infestation index taken from 100 persons of all ages. They are multiplied by 100 in Fig. 7.)

The degree of parasite infestation shows a much more noteworthy rise following the rains. Table XI shows that there is a difference of over 50 per cent. between the lowest and highest infestation indices for the period of investigation. In comparison with Tanga (see Figs. 7 and 8) the rise is relatively less, but this comparison reveals also the much greater total degree of parasitization.

Reference back to Table IX (p. 604) shows, however, that over five-sixths of this parasite load is carried by children under 6 or, put in another way, of 215,000 parasites counted, 186,000 were in children of this age. Similarly, this seasonal rise is chiefly accounted for by a rise in the infestation index among

FIGURE 7.

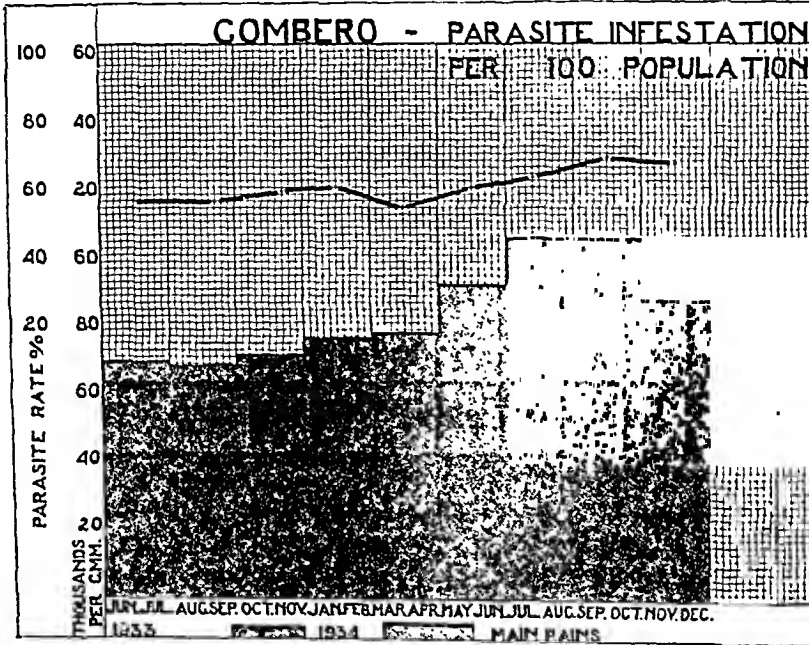
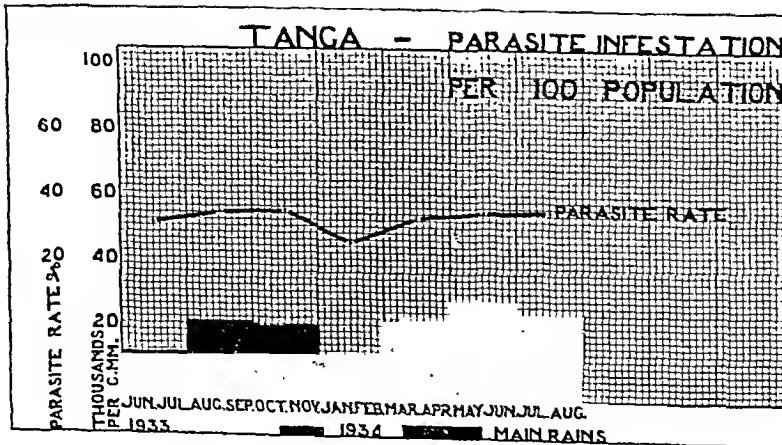


FIGURE 8.



young children : for example, the index in children aged 1 to 5 for September, 1933 was 2,117, while for September, 1934, it was 4,537. The rise becomes progressively less in the older age-groups.

Perhaps in a succession of more normal years this seasonal effect would be less conspicuous, but it would surely in some measure still be present. It reveals the instability of the immunity acquired, even in a highly hyper-endemic area, during the whole of childhood.

5. *Human Infectivity*.—The incidence of gametocytes is summarized in Table XII. From this it is evident that children are by far the most important infectors of mosquitoes. For various reasons gametocytes were not counted separately, but it was found that the numbers present in individual children were very much greater than in adults.

The earliest age at which gametocytes (in this case of *P. falciparum*) have been seen was 6 weeks, but the usual age at which they first appear is 6 to 12 months, and the maximum production is about a year later. At this time gametocytes of all three species may be found, but, corresponding to the general predominance of subtertian infections, crescents are by far the commonest. After the age of 5 years the gametocytes of other species are a rarity.

Some qualification of the figures given in Table XII is necessary. As a result of the practice of examining both thick and thin films, it has been found that "crescents" are quite frequently not seen as such in thick films, and it is only the last four or five series of examinations that are fully affected by this. It has also been found that the quickest way of finding the largest number of gametocytes of all species is by examination of thin films with the $\frac{1}{8}$ inch objective. The combined result of these two improvements in examination is to increase the number of scanty gametocyte infections (mostly adults) by not more than 5 to 7 per cent. (of the number found).

Fig. 9. shows the great difference in gametocyte rates between very young children and the rest of the population, both in Gombero and Tanga. It also shows the greater infectivity in adults accompanied by lesser infectivity in children, of a place where re-infection is not so frequent. Fig. 10 shows a more extreme contrast, in this case with a labour force which included a considerable proportion of very slightly immune people.

Fig. 9 also illustrates the second, and more striking, seasonal change in the blood of children at Gombero. About 3 months after the rains the gametocyte rate, as compared with the dry season, is more than doubled in children, in complete contrast with what happens in adults. This rise is a little later than that in the parasite infestation index. It may also be remarked in passing that the increased infectivity of anopheles is quite independent of the gametocyte rise ; it has, in fact, faded away before the latter reaches its peak.

Substantially, then, the position in these villages is that there are 9 per cent. of gametocyte carriers, of whom two-thirds are children under 5, while the remaining third are carriers of small numbers of crescents.

TABLE XII.
GOMBERO. INCIDENCE OF GAMETOCYTES IN ALL PERSONS EXAMINED.

Date.	Under 5.			6 to 20.			Over 20.			Total per cent.
	Number Examined.	Gameto-cytes.	Gametocytes per cent.	Number Examined.	Gameto-cytes.	Gametocytes per cent.	Number Examined.	Gameto-cytes.	Gametocytes per cent.	
1933										
Apr.	41	9	22.0	98	3	3.1	173	3	1.7	4.8
June	56	14	25.0	88	4	4.5	186	7	3.8	7.6
Aug.	57	13	22.8	93	2	2.2	180	6	3.3	6.4
Oct.	62	18	29.0	92	3	3.3	172	2	1.2	7.1
1934										
Jan.	51	23	45.1	87	9	1.3	189	9	4.8	12.2
Mar.	63	13	20.6	98	3	3.1	188	1	0.5	4.9
May	64	24	37.5	94	3	3.2	182	1	0.6	8.2
July	64	27	42.2	94	6	6.4	180	7	3.9	11.8
Oct.	51	28	54.9	93	11	11.8	185	9	4.9	14.6
Dec.	51	24	47.1	89	14	15.7	168	9	5.4	15.3
Totals	560	193	34.5	926	58	6.3	1,803	54	3.0	9.3

FIGURE 9.

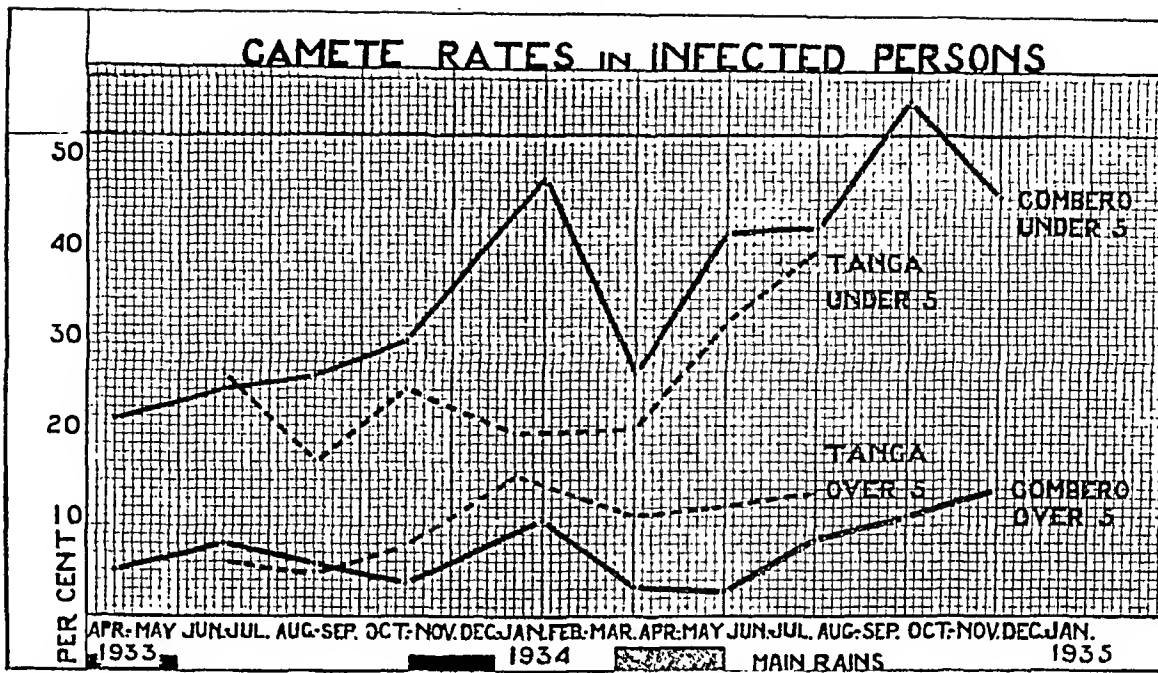
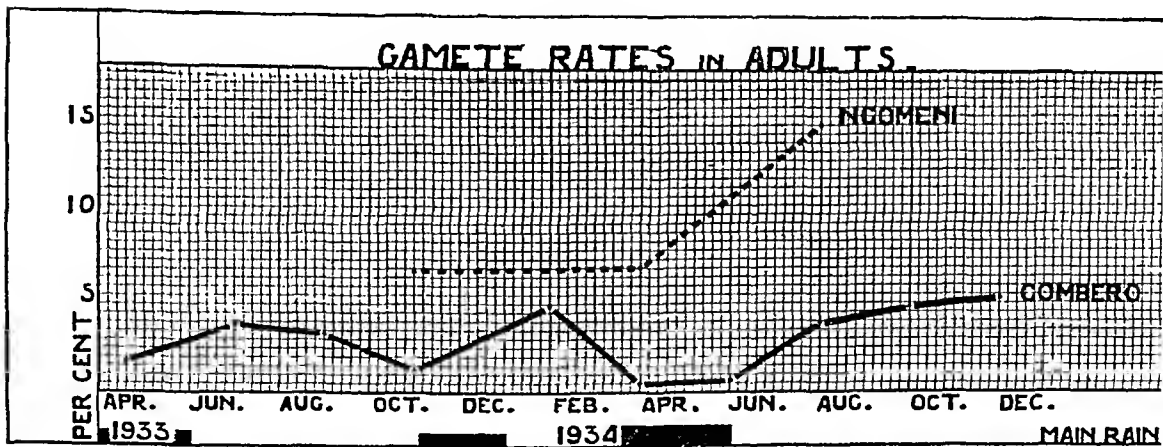


FIGURE 10.



6. *Species Incidence*.—The dominant species of all ages is *P. falciparum*. This is clearly shown in Table XIII, and is in accordance with most other observations in East Africa. While, however, the mean percentage incidence of *P. falciparum* in the whole population is a little over 39.1 per cent. (if allowance be made for the undifferentiated films), there is also a fairly substantial incidence of *P. malariae*, 10.9 per cent., and *P. vivax*, 4.3 per cent. Owing to the fact that they are found chiefly during the earlier years of life, infections with the

two latter species are less common in many other recorded series, more particularly of clinical malaria.

The actual infections at any given age in such an untreated community are the resultant of immunity and re-infection.

TABLE XIII.

GOMBERO. AGE INCIDENCE OF SPECIES. PERCENTAGES TO NUMBER EXAMINED.

Age Group.	Number Examined.	<i>P. falciparum</i> per cent.	<i>P. malariae</i> per cent.	<i>P. vivax</i> per cent.	Undifferentiated.
0- 1	120	82.5	37.5	20.0	
1- 5	393	84.7	41.7	20.1	3
6-10	396	63.4	11.4	4.5	10
11-20	439	39.6	7.5	1.6	32
Over 20	2,045	22.0	4.0	0.9	146
Means		39.1	10.9	4.3	

TABLE XIV.

SPECIES DISTRIBUTION AT GOMBERO, IN POSITIVE AND DIFFERENTIATED FILMS.

Age Group.	<i>P. falciparum</i> .	<i>P. malariae</i> .	<i>P. vivax</i> .	<i>P. falciparum</i> and <i>malariae</i> .	<i>P. falciparum</i> and <i>vivax</i> .	<i>P. falciparum malariae</i> and <i>vivax</i> .	<i>P. malariae</i> and <i>vivax</i> .
0- 1	48	1	1	31	10	19	
1- 5	168	8	3	115	27	64	
6-10	238	11	3	33	14	7	1
11-20	167	12	4	27	2	1	
Over 20	482	55	8	38	10		1
Totals	1,103	87	19	244	63	91	2

P. ovale is included in these tables as *P. vivax*, of which group it forms about a quarter.

It is concluded, therefore, that the immunity acquired to the three species is of a different order in each case. That to *P. vivax* is the most complete, *P. malariae* holds an intermediate position, and immunity to *P. falciparum* is the least complete. The alternative explanation is that there exists a greater

variety of strains of *P. falciparum*, and that, while immunity to one strain is reaching a high titre, infection with a new strain, or with an older strain to which immunity has already largely disappeared, intervenes. Such a multiplicity of strains in one area seems improbable.

The incidence of gametocytes of the three species, in films containing the species in question, was :—

P. falciparum 16·4 per cent., *P. malariae* 14·0 per cent., *P. vivax* 21·6 per cent. It is suggested that the relatively higher rates often given for *P. malariae* are due to the common occurrence of forms whose nature is very difficult to determine, and on the other hand to the failure to recognise young crescents (see p. 609).

P. ovale had an incidence of 0·79 per cent. with a maximum incidence in the age-group 1 to 5 of 1·2 per cent. It has been included in the tables as *P. vivax* for the sake of simplification, but it may be preceded or followed by infections with that species. Gametocytes are present in 34·6 per cent. of positive films, which is oftener than in any other species.

The appearances of *P. ovale* are more temporary than those of the other species, but *P. vivax* is very similar to it in this respect. There may be recurrences of these two species after intervals of several months. *P. malariae* on the other hand is often present in very small numbers for several consecutive examinations, but heavy quartan infections are never seen.

7. *Pyrexia, Anaemia and Malaria*.—The search for a reliable criterion of disability due to malaria has been unfruitful, but two attempts have been made in this direction. Temperatures were taken (for 3 minutes under our direct supervision) thrice daily for a 10-day period in 40 persons of all ages. Of these only three failed to show a temperature of 99° F. or over on one or more occasions. These were a baby of 3 months who had a moderate infection at the period of examination, a child of 2 years and a young adult. Nineteen persons had a temperature of 100° F. or over on one or more occasions. Three persons had temperatures of 101° F. or over, of whom one was a child of 1 year with a moderate malarial infection, and the other two young women, possibly suffering from acute gonorrhoea.

The prime difficulty is the absence of a standard "normal" temperature for Africans, but, even were this available, it seems unlikely that any useful information will be obtained by this method.

The second avenue of approach was by haemoglobin estimations and reticulocyte counts. The results of the former are confirmed by the work of PHILIP (1934) on the same tribe in Kenya.

Table XV shows that in small children under 3 years there is often severe anaemia, with a corresponding reticulocytosis, for which we have failed to find any cause of an importance comparable to malaria. Both schistosomiasis and other helminthic infestations have been definitely excluded at this age. (The earliest schistosome infection was at age 6, and ankylostome at age 2.)

TABLE XV.

GOMBERO. HAEMOGLOBIN AND RETICULOCYTES.

Age.	Haemoglobin.		Reticulocytes.	
	Number Examined.	Per cent.	Per Thousand.	Number Examined.
Under 1	14	53	92.6	10
1	6	56	130.2	6
2	6	61	59.0	12
3	8	68	29.6	8
4	7	70	28.4	9
5	9	73	26.6	3
6-10	42	73	20.1	32
11-15	19	74	15.0	10
16-20	20	78	14.4	17
21-25	29	76	18.6	19
26-30	23	75	20.0	14
31-35	17	76	14.8	14
36-40	24	76	20.8	19
41-50	27	75	11.8	23
Over 50	34	71	21.2	20

The other period at which severe anaemia occurs is in late adult life, when it is almost invariably associated with a high ankylostome infestation. In company with other observers, we have failed to establish any correlation between the degrees of anaemia and ankylostome infestation except in these extreme cases.

Throughout life there is, however, a deficiency of haemoglobin. In newborn babies a haemoglobin percentage of 85 or more was present, but very rarely after this. We are unable to say whether this deficiency is mainly in the quantity or quality of the red cells; but whatever the exact quality of the anaemia, there is a good response to it throughout life. The body has, in fact, become accustomed to the constant drain on its haemoglobin. This is shown by the reticulocyte count, which never drops below about 10 per thousand (*cf.* the European normal of 10 per thousand). Whether that response is adequate for health is another matter.

The average haemoglobin value of 75 per cent. present during adult life indicates a lowered physical efficiency, but can this be attributed to malaria? The number of immature red cells present in the peripheral blood of these people is in the region of 40,000 per c.mm., more often double that. The number of parasitized cells is about 180 per c.mm. In view of this activity of blood regeneration it seems utterly improbable that the anaemia of adult life

can be due to malaria, and its cause must be sought elsewhere. (That cause, we believe, to be ankylostomiasis, coupled with a deficiency of iron in the diet. During adolescence an additional cause of anaemia is schistosomiasis.)

It is concluded that the only period at which the presence of anaemia provides useful indication of the severity of infection and of the damage done by malaria, is during the first two years of life certainly, and during the third year provisionally, subject to the exclusion of ankylostomiasis.

VII.—CONCLUSIONS.

An attempt has been made to present a picture of hyper-endemic malaria in a group of Bantu villages in relation to the circumstances which condition it. These investigations were designed to give some assistance in the formulation of a rationale for native malaria.

They have resulted in the fuller confirmation of some earlier conclusions: that the major issue for such communities is subtertian malaria carried by *Anopheles gambiae*, that a typical decrease in the number and intensity of infections occurs as childhood goes on, and that children are the infectors of anopheles.

But the observations have also added something to the understanding of hyper-endemic malaria. Owing to the length of the period over which the investigation extended, it has been possible to demonstrate that there is very little seasonal difference in the parasite rate, in spite of a great increase in the infected anopheline house infestation; but that there is an increase in the parasite load (mainly in the children) of the community soon after the rains, and that this is followed by a very great increase in the number of gametocytes present in children.

A very high degree of infectivity in the locality is required to produce complete freedom from symptoms in adult life, although the typical decline in frequency of infections may occur, as at Tanga, with a lesser degree of anopheline infestation.

A clear demonstration has been given of the occurrence of the less common species, *P. malariae*, *P. vivax* and *P. ovale*, in children; and the explanation of their non-appearance in non-immunes and their disappearance in fully immunes is in the one case their benignness compared with *P. falciparum*, and in the other the higher degree of immunity achieved by the Bantu to them.

On account both of individual histories and of the incidence of gametocytes in the community at different ages it is postulated that their appearance is neither a sign of immunity nor of its absence, but that they are a sign of the change-over from the non-immune to the semi-immune state, being, in some unknown way, the parasites' response to the throwing-off of an infection. This is in accordance also with the gametocyte wave 3 months after the rains, when semi-immune children are throwing off the effects of more intense infection.

Inherited tolerance plays little part in the ultimate acquisition of immunity, for nearly all babies (after an interval between birth and the first infection) suffered from intense infections which were no less severe than those in other non-immunes such as Europeans. Such inherited characteristics as do play any part in the immune process are rather of the nature of a power to acquire immunity. This is an important distinction in relation to the question of treatment of African babies.

Little light is thrown on the nature of the immune mechanism except by the great initial splenic enlargement, decreasing as mastery over malarial infection is established, and by the reticulocyte response in infants, as evidence of intense activity in erythrocyte regeneration.

There remains the problem of the significance of malaria to such communities. No evidence by physical signs or symptoms, nor estimation of parasites present in the blood, affords any grounds for regarding malaria as an important cause of illness or disability in adult life. The children suffer severely from malaria up to the age of 2, and fair presumptive evidence has been offered that it is an important cause of infantile mortality, either directly or indirectly. But if they have survived this age, they are no longer seriously affected by infections which are only a quarter or a fifth of the intensity of those they have successfully passed through. And it cannot be supposed that, during and after adolescence, infections of a fiftieth of their infantile intensity can play any important part in the causation of the anaemia, infertility, lack of vigour, nor of the decline after adolescence, which are so commonly attributed to malaria.

Of these two conclusions, on the one hand the danger of malaria to infants, and on the other its harmlessness in youth and adult life, the second is unfortunately, but I believe inevitably, based on negative evidence. Yet they are the two sides of the medal, and they form together the essential basis for work in African villages in the tropics.

SUMMARY.

1. There is a real need for a detailed study of hyper-endemic malaria in African villages, in order to provide the necessary background to more summary observations in other parts.

2. A survey of previous work shows that, while a few careful studies of this nature have been made in other parts of the world, Africa is not so fortunate. The previous observations recorded show that the findings in different countries cannot be fitted into a coherent whole.

3. A description is given of the people studied, their diet, and their conditions of life, with some indication of the diseases to which they are subject and of the birth and death rates.

4. There is a high prevalence of *A. gambiae* in houses, and a mean sporozoite infectivity of 12.2 per cent. The infectivity has a seasonal rise to 24 per cent., at a time when there is an average of nearly 15 female anopheles to a house.

5. In a detailed clinical description of the course of malarial infection in this untreated population, it is shown that all babies are infected within 5 months of birth, and that all have severe infections.

6. This stage of acute infestation has passed its zenith after 18 months, and is succeeded by a period of malarial instability, during which the parasite count rises and falls irregularly. But symptoms are absent.

7. After puberty symptoms are completely absent, and the general parasite level is a fiftieth or less of that which is present during infancy.

8. The spleen rate is high, being 85 per cent., but it decreases with the parasite rate, as immunity is acquired. The largest spleens are found in babies soon after their first infection.

9. There is a seasonal rise in the parasite infestation of man, which is chiefly confined to the non-immune and partially immune children.

10. Gametocytes are found predominantly in children, at the time when immunity is just beginning to be established. Even if they are present in adults, they occur in very small numbers only. There is a great seasonal rise in the frequency and number of gametocytes in children under 5 years of age.

11. *P. falciparum* is the dominant infection at all ages, but the other species (including *P. ovale*) are also present during the first few years of life, and more rarely later. The immunity acquired to *P. falciparum* is less complete than that acquired to the other species.

12. While children suffer from malaria during the first 2 years of life, and a few die during the first few months, older children cannot be said so to suffer, and no evidence was obtained of any harmful effects from malaria during adult life.

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MORPHOLOGICAL AND TAXONOMIC STUDIES ON MAMMALIAN TRYPANOSOMES.

II. *Trypanosoma simiae* AND ACUTE PORCINE TRYPANOSOMIASIS IN TROPICAL AFRICA.

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I.—INTRODUCTION.

Amongst the livestock in tropical Africa, the pig (*Sus scrofa*) appears to be the least subject to infection by the trypanosomes pathogenic to other domestic animals.

The only species commonly found in the domestic pig is *Trypanosoma congolense*, which causes a chronic infection without appreciably impairing the health of the animal. As far as could be ascertained, there are only three records of natural infection of pigs with *T. brucei*, one by BOUET (1908) who saw two cases in Dahomey, another by MACFIE (1915) who found one infected pig out of a hundred examined in the slaughter-house at Accra, Gold Coast, and the third by STEWART (1935), also from the Gold Coast, who reported sixteen cases of infection due to exposure of pigs to tsetse flies. In none of these cases were any symptoms mentioned and it is probable that the pigs were merely "carriers."

However, in a number of publications *T. brucei* has been credited with being very pathogenic to pigs. This view is evidently based on the experimental infections carried out in 1902 by LAVERAN and MESNIL and by BRADFORD and PLIMMER, on the one hand; and on the isolated outbreaks of acute porcine trypanosomiasis, attributed to *T. brucei*, which were described by OCHMANN (1905), LICHTENHELD (1912) and ALDIGÉ (1920), on the other hand.

In the case described by LAVERAN and MESNIL (cf. 1904 and 1912), a piglet inoculated with *T. brucei* lived for 94 days without showing any symptoms of disease during the first 40 days, or any trypanosomes in the blood until 2 days before its death. According to BRADFORD and PLIMMER (1902), "the pig shows the organism in the blood very rarely and in very small numbers, and dies with pulmonary symptoms." It is thus seen that the experimental infections in the pig ran a chronic course, and the available evidence is not sufficient to incriminate this trypanosome in the pathological symptoms observed. As to the outbreaks of acute disease, it will be shown later that they were in all probability due to a different species of trypanosome.

To sum up, it would appear that, until satisfactory evidence to the contrary is forthcoming, the pig can be regarded as being relatively resistant to infection with *T. brucei*.

The occurrence of *T. uniforme* in pigs was reported on one occasion (HORNBY, 1923) but, as will be demonstrated below, the disease with which it was associated cannot be attributed to this trypanosome.

In addition to the records of the occurrence of the trypanosomes referred to above (*T. congolense*, *T. brucei*, *T. uniforme*) which are either harmless to the pig or cause a mild chronic disease, there have appeared from time to time, and especially during the last decade, reports of outbreaks of an extremely acute form of trypanosomiasis amongst pigs in various parts of tropical Africa. These outbreaks have so many features in common that a summary description of the typical picture can serve to characterize them all. The onset of disease is always sudden: a pig which may have been apparently healthy a day or even several hours earlier, suddenly falls ill, becomes progressively worse and dies within the next few days, but in exceptional cases the course of the disease may be prolonged. Owing to the rapid development of the disease there are no noteworthy symptoms, but trypanosomes are invariably swarming in the blood. The infection spreads rapidly through the herd, killing the pigs in large numbers within a very short time, and before any precaution can be taken or treatment carried out. The sporadic occurrence of these outbreaks and rapid course of the disease are responsible for the lack of any detailed observations on the clinical aspects and method of propagation of the infection. As regards the causative agent, with which we are primarily concerned here, its exact nature has so far baffled every observer, with the result that the various outbreaks have been attributed to at least seven different trypanosomes, of which three have been described as new species (*T. suis*, *T. brucei*, *T. vivax*, *T. uniforme*, *T. rodhaini*, *T. porci* and *T. simiae*).

The serious nature of this disease and the unsatisfactory state of our knowledge regarding its causative agent have recently given rise to some concern and were discussed at the "Conference on Tsetse and Trypanosomiasis Research" held at Entebbe in 1933, and by the "East Africa Sub-Committee of the Tsetse Fly Committee" in London (cf. Report, 1935). Both these bodies recognized

the importance of elucidating the aetiology of acute trypanosomiasis in pigs and included it in the suggested programme of investigations to be undertaken in East Africa.

Having been engaged for some time in the systematic study of various mammalian trypanosomes, I decided to include the trypanosomes responsible for the outbreaks among pigs in my programme of work, in the hope of throwing more light on their identity.

I was fortunate enough to obtain a large number of blood films from infected pigs which were very kindly placed at my disposal by the following: Prof. J. SCHWETZ, lately Director of the Parasitological Laboratory at Stanleyville, Belgian Congo; Dr. G. C. BOURGUIGNON, Director of the Bacteriological Laboratory at Elisabethville, Belgian Congo (through the kind offices of Prof. J. RODHAIN, of the Institute of Tropical Medicine at Antwerp); Dr. G. NORMAN HALL, of the Nigerian Veterinary Service; and Prof. J. G. THOMSON, of the London School of Hygiene and Tropical Medicine.

I take this opportunity of expressing to these gentlemen my deepest gratitude for the generous way in which they have responded to my request, by placing their material—which in the case of Drs. SCHWETZ and BOURGUIGNON had served for their own published investigations—unreservedly at my disposal. Needless to say that but for their assistance it would not have been possible to carry out this investigation.

II.—RECORDS OF OUTBREAKS AMONG PIGS.

Before proceeding with my own observations, I propose to review briefly all the records of acute trypanosomiasis in pigs which I have been able to trace (see Map, p. 623).

The first account was given by OCHMANN (1905) who observed several cases of fatal trypanosomiasis of short duration in Dar-es-Salaam. The blood of the pigs contained numerous trypanosomes which were said to be shorter and thicker than *T. brucei* and for which the name *T. suis* was tentatively proposed.

Later LICHTENHELD (1912) described three outbreaks among pigs in German East Africa in which the course of the disease was so violent ("stürmisch") that the animals rapidly succumbed without exhibiting any special symptoms. As in the previous case trypanosomes were extremely numerous in the blood. The author attributed the outbreaks to nagana, apparently without having critically studied the trypanosomes themselves. It is noteworthy that equines and bovines kept in the neighbourhood of the affected pigs did not contract the infection.

The next outbreak was observed in Southern Rhodesia by BEVAN (1917) who stated that "a large number of pigs were dying from some unknown disease at two farms on the northern border of the Umfuli River." Blood films from the sick animals "revealed large numbers of trypanosomes of *pecorum* [= *congolense*] group, but differing slightly morphologically from the trypanosome commonly met with in cattle . . . , notably in the fact that the body appeared

more flexible and the undulating membrane more highly festooned." Inoculation of the blood from infected pigs failed to produce an infection in small laboratory animals, or in cattle and sheep, but in pigs it set up "a disease rapidly fatal, killing untreated animals in less than thirty days."

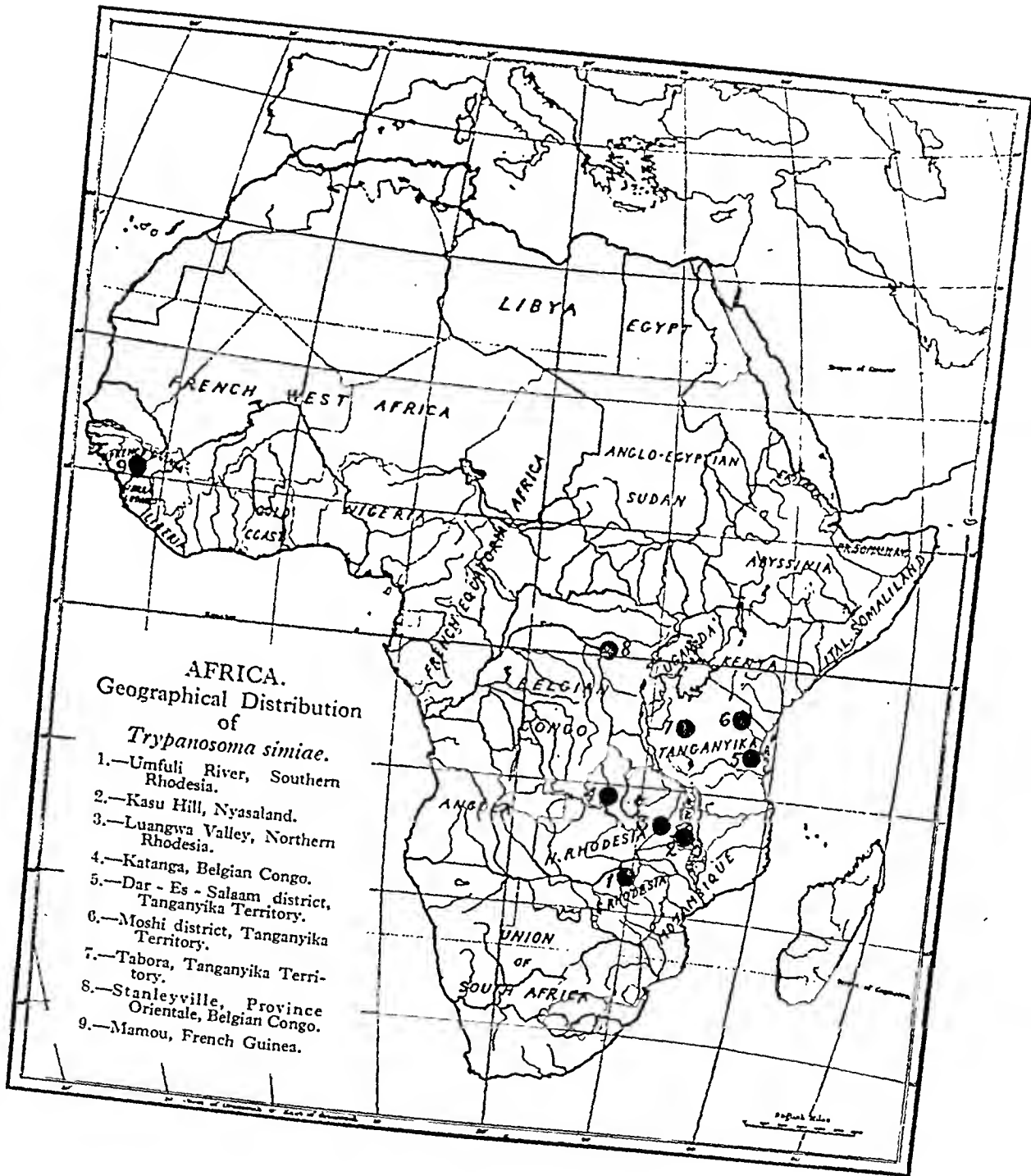
Another outbreak was reported from French Guinea by ALDIGÉ (1920) who noted the extremely rapid development of the disease ("presque foudroyante"), killing off the pigs within from 12 to 48 hours. Trypanosomes were again so abundant that the blood was said to be like a pure culture of these flagellates. The parasite was referred to *T. brucei* with some doubts, on account of the presence of atypical short forms, 12–14 μ long and possessing a short flagellum. This is the only record from West Africa.

I am indebted to Dr. G. N. HALL for communicating to me particulars of an outbreak observed by him in 1920, near Moshi, Tanganyika Territory. The infection and mortality in this instance were heavy, affecting the majority of the pigs on a farm. The trypanosomes, which were numerous, were thought to be *T. uniforme*.

Another outbreak of severe and fatal trypanosomiasis was reported from Tabora, in the same colony, by ARMFIELD (1922) and by HORNBY (1924). One pig died "after only four hours' illness" and was found to harbour numerous trypanosomes, while two other pigs which died under similar conditions were not examined. The trypanosome was at first identified as *T. vivax*, but later HORNBY (1923) referred it to *T. uniforme*.

The most numerous epizootics have occurred during the last decade or so in the Belgian Congo. The first of these were recorded from Katanga by WALRAVENS alone (1924, 1926, 1927), and in collaboration with others (WALRAVENS *et al.*, 1927). The trypanosome responsible for this fatal disease which, according to SCHWETZ (1934), decimated the pigs was regarded as a new species to which the name *T. rodhaini* was given. Its main characteristics are a very slender elongated body, an undeveloped undulating membrane, and the presence of a fairly long free flagellum, while the kinetonucleus is invariably marginal. The trypanosome was found to be highly virulent for monkeys (*Cercopithecus* sp.), but guineapigs, rabbits and a bull were refractory. WALRAVENS' views were criticized by HORNBY (1926) who identified *T. rodhaini* with *T. uniforme*.

Observations in the Belgian Congo were continued by SCHWETZ (1930, 1932, 1934) who described a series of outbreaks from the vicinity of Stanleyville. The disease exhibited the typical symptoms noted in the Introduction (p. 620). Among the trypanosomes, which were always present in large numbers in the blood of the infected animals, SCHWETZ noted a variety of forms exhibiting the structure of *T. congolense*, *T. simiae* and *T. rodhaini*. After some hesitation this observer (SCHWETZ, 1930) concluded that he was dealing with a polymorphic trypanosome of the *congolense* group which he at first regarded as a distinct species and named *T. porci* (SCHWETZ, 1932), but later as a subspecies of *T. congolense* [= *T. congolense porci*] (SCHWETZ, 1934). Experimental infections



AFRICA.
Geographical Distribution
of
Trypanosoma simiae.

- 1.—Umfuli River, Southern Rhodesia.
- 2.—Kasu Hill, Nyasaland.
- 3.—Luangwa Valley, Northern Rhodesia.
- 4.—Katanga, Belgian Congo.
- 5.—Dar - Es - Salaam district, Tanganyika Territory.
- 6.—Moshi district, Tanganyika Territory.
- 7.—Tabora, Tanganyika Territory.
- 8.—Stanleyville, Province Orientale, Belgian Congo.
- 9.—Mamou, French Guinea.

sometimes succeeded in sheep, but failed in rats, guineapigs, a dog, a goat, a calf and in monkeys (*Colobus*, *Cercocebus*, *Cercopithecus*).

Further outbreaks in the Belgian Congo were described by BOURGUIGNON (1933, 1935*) and BOURGUIGNON and JUSSIANT (1934) from Stanleyville and from Katanga. The symptoms of the disease were similar to those described by SCHWETZ, but in four cases its course was more protracted. Trypanosomes were always abundant in the blood of the pigs. Like SCHWETZ, BOURGUIGNON arrived at the conclusion that the causative agent was a polymorphic trypanosome with a range of forms including the *congolense*, *simiae* and *rodhaini* types of structure. He did not, however, accept SCHWETZ's *T. porci*, but regarded the trypanosome as being *T. simiae*, though admitting that it did not conform to the classical type of this species. In BOURGUIGNON's opinion, the polymorphism of *T. simiae* is based mainly on the presence or absence of a free flagellum, the other morphological forms present being treated as individual variations.

As to *T. rodhaini*, in one paper (BOURGUIGNON, 1935), it is suggested that it represents a morphological variety of *T. simiae* [= ? *T. simiae* var. *rodhaini*], while in a later paper (BOURGUIGNON, 1935a) it is described as a valid species, under its original name, and is assigned to the *vivax*-group (cf. HORNBY, 1926). BOURGUIGNON inoculated a number of guineapigs with the porcine trypanosome, with negative results.

The records of outbreaks of acute porcine trypanosomiasis reviewed above are, as far as I am aware, all that have been published. Only the most important data on the nature of the causative trypanosomes, as conceived by the observers themselves, are given here, their critical examination being reserved for other sections of this paper.

Before concluding this section it should be pointed out that the typical symptoms of the disease exhibited in the outbreaks coincide exactly with those observed by BRUCE and his collaborators (1913, 1913b, 1915) in experimental infections of the pig with *T. simiae*. This trypanosome is graphically described as "the lightning destroyer of the domestic pig," and it is stated that "In the whole range of the trypanosome diseases of animals there is surely nothing so striking as the rapidly fatal action of *T. simiae* on the domestic pig. In nine experiments the average duration was only 5.3 days . . . from the date of infection . . . In regard to the symptoms of the disease during life, nothing noteworthy happens owing to the rapidity of the disease."

III.—IDENTITY OF THE PIG-TRYPANOSOME.

1.—GENERAL MORPHOLOGY.

The outline given in the preceding section shows clearly the confusion and uncertainty regarding the causative organism of acute porcine trypanosomiasis.

* This paper was read before the Société Belge de Médecine Tropicale in 1934. I am indebted to Dr. BOURGUIGNON and Professor RODHAIN for placing the MS. copy at my disposal before its publication.

On the other hand, it brings out the striking similarity in the various accounts of the disease, reported from different parts of Africa by observers working independently and, in most cases, in ignorance of each other's findings.

While the existing records apparently point to a multiplicity of causative agents, the marked uniformity of the clinical manifestations naturally suggests a common origin of these outbreaks, and it is with the object of elucidating this problem that this study was undertaken.

It is based, on the one hand, on direct examination of preparations from infected pigs, and, on the other hand, on a critical analysis of the literary data.

The material at my disposal consisted of the following blood films from infected pigs (stained by the Romanowsky method):—

(1) *T. rodhaini*: Dr. WALRAVEN's slides, from Katanga, Belgian Congo (obtained from Dr. SCHWETZ).

(2) *T. porci*: slides from Dr. SCHWETZ, from cases obtained in the neighbourhood of Stanleyville, Belgian Congo.

(3) *T. simiae*: slides from Dr. BOURGUIGNON, from cases obtained in Katanga, Belgian Congo.

(4) "*T. uniforme*": slides from Dr. HALL, from cases observed by him in Moshi, Tanganyika Territory.

(5) *T. simiae*: one of the original films made by Sir DAVID BRUCE in Nyasaland (lent by Professor THOMSON).

A preliminary examination of the available films soon revealed that they were not all equally suitable for an exact morphological study, the trypanosomes in a large proportion of them being in such a defective state that the preparations had to be rejected. A detailed study was, however, made of the slides in which the trypanosomes were well preserved. Nevertheless, the imperfect films proved to be useful in other ways: while the normal trypanosomes occasionally present in them served for comparison with those in the good films, the defective trypanosomes provided clues for the correct interpretation of the discrepancies occurring in the writings of some of the authors.

On careful examination of the films from the Congo (Dr. SCHWETZ's and Dr. BOURGUIGNON's cases) and from Tanganyika (Dr. HALL's cases) it was found that the trypanosomes from all these sources exhibited a similarity of structure and of form, which left no doubt that they all represented the same organism.

In view of this, it is possible to describe them collectively. The predominant forms of trypanosomes in all these cases do not differ in any respect from the typical *T. simiae*, as described by BRUCE and his collaborators (1912) (Fig. A, 1-5, 10-13, 17-20). They represent elongated trypanosomes, with a well-developed undulating membrane usually extending to the very tip of the flagellum; the kinetonucleus is subterminal and situated at the margin of the body, the posterior end of which is rounded or bluntly pointed. In addition to trypanosomes of the *simiae*-type there occur slender elongated forms with a weakly

developed undulating membranc (Fig. A, 6, 7, 14, 21). These forms show a certain resemblance to the trypanosome described by WALRAVENS and will, therefore, be referred to as *rodhaini*-like forms. In both the *simiae*- and *rodhaini*-like forms some individuals appear to have a free flagellum (Fig. A 13). The third type of trypanosome encountered in the strains under consideration is indistinguishable from *T. congolense* (Fig. A, 8, 15, 23, 24) : it represents a relatively short trypanosome (as compared to the other two types) with an undeveloped undulating membrane extending to the tip of the flagellum. On single occasions I have encountered aberrant forms, such as "ablepharoplastic" forms (Fig. A, 9), and one or two like *T. uniforme*, except for the absence of a free flagellum and the small kinetonucleus (Fig. A, 16).

My observations thus confirm the findings of SCHWETZ and BOURGUIGNON as regards the polymorphic nature of the pig-trypanosome, and extend them to the case observed by HALL. Both SCHWETZ (1930-1934) and BOURGUIGNON and JUSSIANT (1934) noted that the characters of the trypanosomes observed by them do not fit in with any one known species, but rather represent the united characters of at least three species :—*T. simiae*, *T. congolense* and *T. rodhaini*. The Belgian authors actually considered the possibility of the polymorphism of the pig-trypanosome being due to mixed infections (BOURGUIGNON, 1933 ; SCHWETZ, 1934), but this view was subsequently abandoned, SCHWETZ (1932, 1934) regarding the trypanosome as a new species or subspecies (*T. porci* or *T. congolense porci*) because of its polymorphism, while BOURGUIGNON and JUSSIANT (1934) identified it as *T. simiae* in spite of its polymorphism.

T. simiae, according to BRUCE *et al.* (1912), is "monomorphic and, as a rule, fairly uniform in shape," and this definition of the parasite is borne out by their illustrations. The only other description of *T. simiae* (under the name *T. ignotum*), given by KINGHORN and YORKE (1912), is entirely in agreement with the preceding one. As stated already, the pig-trypanosome from the Congo and from Tanganyika differs from the classical *T. simiae* in its polymorphism, a feature which would fully justify the recognition of its independent status, if not as a species, at least as a race or variety. The position of *T. rodhaini* Walravens, 1924, will be discussed later.

In order, if possible, to throw more light on the relationship of the pig-trypanosome to *T. simiae*, I made a careful study of one of Sir DAVID BRUCE's original films. The examination resulted in establishing the presence in it both of *rodhaini*-like and *congolense*-like forms (Fig. A, 25-28). These forms, being in a minority, were probably disregarded by BRUCE and his co-workers, who based their description exclusively on the predominant type of trypanosome. They note, however, in another paper (BRUCE *et al.*, 1913) that "it is often difficult or impossible to distinguish between a short individual of . . . [*T. simiae*] and a long one of . . . [*T. congolense*]." The discovery of polymorphism in the original *T. simiae* thus removes the only objection to regarding the pig-trypanosome as a true *T. simiae*. It should be added that there is every gradation

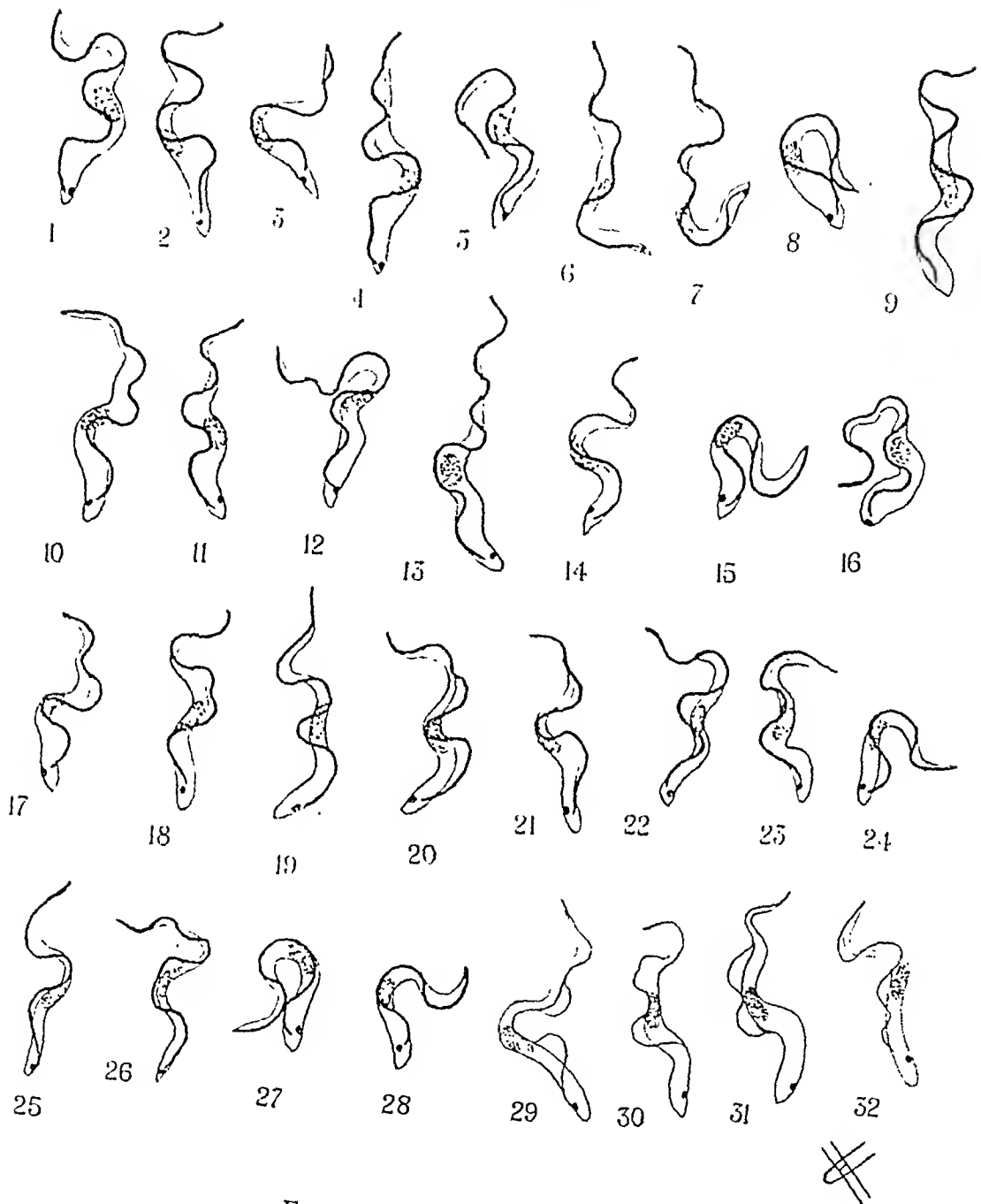


FIG. A. *Trypanosoma simiae*.

1 to 9 : case "CM," Stanleyville, Belgian Congo (Dr. SCHWETZ's preparation) ;
 10 to 16 : case "C447," same locality (Dr. SCHWETZ's preparation) ;
 17 to 24 : case "10f," Katanga, Belgian Congo (Dr. BOURGUIGNON's preparation) ;
 25 to 28 : case from Nyasaland (Sir DAVID BRUCE's preparation) ;
 29 to 32 : "*T. rodhaini*" (Dr. WALRAVEN's preparation : film defective).
 1, 2, 3, 10, 11, 12, 17, 18, 29, 30, 31, 32—predominant (standard *simiae*) forms ; 4, 5,
 13, 19, 20—forms with free flagellum ; 6, 7, 14, 21, 22, 25, 26—slender ("*T. rodhaini*"-like)
 forms ; 8, 15, 23, 24, 27, 28—"*T. congolense*"-like forms ; 9—"ablepharoplastic" form ;
 16—atypical form, resembling *T. uniforme*.
 (All the figures were drawn with the aid of a camera lucida, at $\times 2,000$.)

between the various morphological types of *T. simiae*, their separation into three groups being based on the extreme forms of each type.

It remained to be seen whether a quantitative study of all the strains of the pig-trypanosome at my disposal corroborated their unity and their identity with *T. simiae*. In the first place, I selected some of the best films (Dr. SCHWETZ's cases "CM" and "C477," Dr. BOURGUIGNON's "10F" and Dr. HALL's case) and measured the length of 100 individuals in each.* The results obtained are set forth in Table I, together with the data of other authors, for comparison.

From Table I it is seen that the dimensions of the pig-trypanosome from the Congo and the Tanganyika strains closely agree not only with each other, but also with the measurements of *T. simiae* made by BRUCE *et al.*, and KINGHORN and YORKE, especially in the mean lengths which vary from 17.0μ to 18.2μ . The mean lengths given by BOURGUIGNON and JUSSIANT for two strains, including the one measured by myself ("10F"), are somewhat higher, but this is probably due to a difference in the total number of individuals measured.

The next point was to determine the proportion of the various forms constituting the polymorphism in the pig-trypanosomes and in BRUCE's *T. simiae*. For this purpose 500 individuals in each strain were sorted into three groups according to whether they were of the original *simiae*-, *rodhaini*- or *congolense*-types of structure. The only previous attempt to group the forms seen in the pig-trypanosome was made by SCHWETZ (1934). The results of this count are given in Table II, which includes SCHWETZ's figures.

Table II brings out the remarkable uniformity of all the strains as regards the proportion in which the three morphological types (*simiae*, *rodhaini*, *congolense*) occur in each of them. However, SCHWETZ's figures show a much larger percentage of *rodhaini*-like forms, an explanation of which will be given later (p. 632), when dealing with WALRAVENS' *T. rodhaini*. It might be argued, as it was by SCHWETZ and BOURGUIGNON at one time, that the polymorphism of the pig-trypanosome is due to mixed infection. However, the following facts provide strong arguments against such an interpretation: first, the presence of intermediate forms which unite the recognized three types into a continuous series; secondly, the presence of these forms in the same numerical proportion in all the strains examined. The regular occurrence of the three forms was noted by BOURGUIGNON and JUSSIANT (1934) in all the cases of pig-trypanosomiasis observed by them in the course of three months.

The observations on the morphology of the trypanosomes recorded above have established the following facts: (1) that the original *T. simiae* was not a monomorphic trypanosome, as stated by BRUCE *et al.* (1912), but polymorphic; (2) that the various strains of pig-trypanosomes from the Congo and Tanganyika

* The trypanosomes were taken in the order in which they appeared in view, and the measurement was made by drawing (with the aid of a camera lucida at $\times 2,500$) a line running through the middle of the body of the trypanosome from the posterior end of the body to the tip of the flagellum, and then measuring this line with a divider set at $0.5\mu \times 2,500$.

TABLE I.
MEASUREMENTS OF *Trypanosoma simiae*.

Observers.	Strains.	Total length of trypanosomes in microns.			Length of free flagellum in microns.	No. of individuals measured.
		Minimum.	Mean.	Maximum.		
HOARE	<i>T. porci</i> " CM " (Belgian Congo)	13.0	17.0	21.5	1.7-2.7	100
"	<i>T. porci</i> " C477 " (Belgian Congo)	12.5	17.4	22.7	1-3.5	100
"	<i>T. simiae</i> " 10F " (Belgian Congo)	13.5	17.0	20.0	1-1	100
BRUCE <i>et al.</i> (1912)	" <i>T. uniforme</i> " (Tanganyika)	14.0	18.2	21.5	1-3.5	100
KINGHORN and YORKE (1912)	<i>T. simiae</i> (Nyasaland)	14.0	17.5	24.0	1-3	500
WALRAVENS (1926)	<i>T. ignotum</i> (N. Rhodesia)	12.0	17.0	23.0	?	200
BOURGUIGNON and JUSSIANT (1934)	<i>T. rodhaini</i> (Belgian Congo)	15.0 ¹	?	20.0 ¹	4.2-12.5	100
"	<i>T. simiae</i> " 10F " (Belgian Congo)	?	18.9	?	?	?
"	<i>T. simiae</i> " 14A " (Belgian Congo)	?	19.7	?	?	?

¹Compiled according to the scale accompanying the figures; in the original only the body length, without flagellum, is given.
²Some authors give the mean total length of this trypanosome as 19.8 μ , obtained by adding mean body length to mean length of flagellum (actually = 19.62 μ) which is an entirely erroneous procedure.

TABLE II.
DEGREE OF POLYMORPHISM IN *Trypanosoma simiae*.
(PER CENT.)

Observers.	Strains.	<i>simiae</i> -like forms.	<i>rodhaini</i> -like forms.	<i>congolense</i> -like forms.	No. of individuals counted.
HOARE	Dr. BOURGUIGNON's " 10F " (Belgian Congo)	88.8	7.8	3.4	500
"	Dr. SCHWETZ's " CM " (Belgian Congo)	88.8	7.0	4.2	500
"	Dr. HALL's (Tanganyika)	92.2	5.2	2.6	500
"	Sir D. BRUCE's (Nyasaland)	91.4	6.2	2.4	500
SCHWETZ (1934)	Pig 1 (Belgian Congo)	75.7 ¹	20.3	4.0	?
"	" 6 (" ")	71.7	24.0	4.3	?
"	" 477 (" ") ²	58.0	22.5	9.5	?

¹The figures in this column include the forms regarded by SCHWETZ as intermediate between the *simiae*-like ones and the others (*congolense*-like and *rodhaini*-like).

²10 per cent. of trypanosomes in this strain are described as " involution " forms.

are morphologically indistinguishable from each other and from *T. simiae* as amended here; (3) that, therefore, the pig-trypanosome is identical with *T. simiae*.

It should be emphasized here that during the twenty odd years that have elapsed since the discovery of *T. simiae* only SCHWETZ and BOURGUIGNON have considered its possible association with the disease naturally occurring in pigs: the present investigation has established this connexion beyond doubt. Hitherto the infectivity of *T. simiae* to the pig, amongst other domestic animals, was known only from the experimental infections carried out by BRUCE's Commission.

2.—STATUS OF *T. rodhaini*.

WALRAVENS (1924, 1926) gave the name *T. rodhaini* to a trypanosome responsible for outbreaks of fatal disease in pigs with symptoms like those produced by *T. simiae*. The trypanosome was said to be monomorphic, with characters already described above (p. 622). The total length of the trypanosomes is not given in WALRAVENS' paper, but the ones illustrated in his figures measure

from 15μ to 20μ , as calculated from the scale. The only known trypanosome to which *T. rodhaini*, as described by WALRAVENS, could be likened is *T. uniforme*, and this was done by HORNBY (1926), who referred WALRAVENS' trypanosome to the latter species, regarding it as identical with the trypanosome found in the outbreak described by ARMFIELD (1922) and by himself (HORNBY, 1924).

However, in his reply to HORNBY's criticism, WALRAVENS (1927) emphasized the extreme slenderness of *T. rodhaini* (maximum breadth = 0.9μ) as a character distinguishing it from *T. uniforme* (maximum breadth = 2.5μ). In their strains of the pig-trypanosome, SCHWETZ (1930, 1934) and BOURGUIGNON and JUSSIANT (1934) always found forms similar to *T. rodhaini* in association with trypanosomes of the *simiae*- and *congolense*-types, and both SCHWETZ (1934) and BOURGUIGNON (1935) admitted the possibility of *T. rodhaini* being an independent variety. BOURGUIGNON (1935) at first attached this variety to *T. simiae*, but later he (BOURGUIGNON, 1935a) apparently changed his views, and treated *T. rodhaini* as an independent species belonging to the *vivax*-group. It should be added that SCHWETZ (1934) did not regard *T. rodhaini* as strictly monomorphic, having noted some difference in the length of the body and of the flagellum between the pig and monkey strains of this trypanosome. However, judging from the figures (*loc. cit.*, Pl. II, Fig. 4) this difference is quite negligible and not essential.

From the descriptions of *T. rodhaini* given by WALRAVENS and by SCHWETZ it appeared to differ from the pig-trypanosome, now identified with *T. simiae*, sufficiently to be regarded at least as a distinct variety. However, an examination of two of WALRAVENS' films at my disposal soon changed my opinion. Both these preparations proved to be defective, the films being excessively thick and their staining very poor. The body of the trypanosomes was very slender with faint outlines and without any visible undulating membrane. The flagellum was indistinct in the majority of specimens, while in some there were indications of a short free flagellum. The extent of the latter could not be definitely ascertained owing to the fading away of the body towards the anterior end. However, on carefully searching the preparations isolated areas were discovered in which the staining was somewhat better, and in these the trypanosomes exhibited all the attributes of *T. simiae*: a broad body, with a well-developed undulating membrane extending to the tip of the flagellum, as depicted in Fig. A, 29-32. The kinetonucleus of WALRAVENS' trypanosome is small and usually situated at the margin of the body. The small size of the kinetonucleus alone is sufficient to distinguish *T. rodhaini* from *T. uniforme* with which HORNBY (1926) confused it.

The presence of typical *simiae*-forms and of the characteristic agglutinated couples, to be described later, proves conclusively that WALRAVENS' *T. rodhaini* is nothing else but *T. simiae* as it appears in badly prepared blood films. The dimensions of "*T. rodhaini*" also fall within the range of *T. simiae* (cf. Table I).

The slenderness of the body, the flatness of the undulating membrane, and the indistinct flagellum in "*T. rodhaini*" were obviously due to defects in the films, the slenderness being the result of contraction of the body usually occurring

in thick films. Except for the appearance of the flagellum, the description of "*T. rodhaini*" given by WALRAVENS agrees with the picture seen by me, leaving no doubt in my mind that he had relied in his observations on similarly defective films.

It has been mentioned already (p. 625) that a large proportion of the films from the Belgian Congo examined by me proved to be unfit for an exact morphological study. The trypanosomes in these films showed the same defects as those of WALRAVENS and the predominant forms in them were similar to his "*T. rodhaini*." The occurrence of defective forms also explains the high percentage of *rodhaini*-like forms recorded by SCHWETZ (1934) for some of his strains (cf. Table II).

That a small percentage of slender, "*rodhaini*"-like forms normally occur in perfect preparations of *T. simiae* has already been demonstrated above.

3.—FREE FLAGELLUM IN *T. simiae*.

The question of the presence or absence of a free flagellum in *T. simiae* has never been properly established. According to BRUCE *et al.* (1912), "it is difficult to say whether this species has a free flagellum or not." In most cases, the undulating membrane extended to the tip of the flagellum, but "the last two or three microns of the flagellum often appear to be free." KINGHORN and YORKE (1912) state more precisely that "a short flagellum is only very occasionally present." In the works of WALRAVENS (1926), SCHWETZ (1934), BOURGUIGNON (1935) and BOURGUIGNON and JUSSIANT (1934) the presence of a free flagellum in a certain proportion of the trypanosomes is quite definitely recognized.

WALRAVENS' views on this point are of little value, since they were based on defective preparations in which the extent of the undulating membrane cannot be ascertained. An examination of all the films at my disposal has shown, as a general rule, that the number of forms which appear to have a free flagellum is directly proportional to the imperfection of the preparation examined. The minute details of structure can only be appreciated in faultless preparations, and these exclusively were used by me in studying the morphology of the pig-trypanosome.

A careful study of various strains of *T. simiae* revealed the following facts. In the short *congolense*-like forms (Fig. A, 8, 15, 23, 24, 27, 28) the flagellum is never free, the body extending quite definitely to the tip of the flagellum. In the long forms (standard *simiae*-type and *rodhaini*-like forms) it is sometimes difficult to establish the true relations owing to the extreme attenuation of the undulating membrane in the anterior quarter or third of the body. However, with good illumination the undulating membrane is seen to extend to the very tip of the flagellum in the great majority of individuals. This is especially clear in specimens in which the terminal portion of the flagellum does not cross over to the opposite side of the body (Fig. A, 3, 6, 10-12, 21, 22, 25). In specimens in which this "crossing over" takes place, it may be difficult to decide

whether the membrane reaches the end of the flagellum or not. Sometimes it is clearly visible (Fig. A, 7, 14), at other times it is invisible (Fig. A, 5, 19, 26). In the latter case it is conceivable that the twist of the flagellum causes the corresponding portion of the undulating membrane to lie vertically in relation to the plane of the film, with the result that its outline coincides with that of the flagellum which therefore appears to be free. The plausibility of this explanation rests on the fact that in all the specimens in which the end of the flagellum is not twisted it is accompanied by the undulating membrane throughout its entire length. It is likewise difficult to decide whether the flagellum is free in specimens in which the anterior part of the membrane is so narrow as to merge imperceptibly with the flagellum. Finally, in a small proportion of specimens the membrane does not appear to reach the end of the flagellum, and these are the only ones, in my opinion, in which the presence of a free flagellum can be admitted more or less unreservedly (Fig. A, 4, 13). The free portion of the flagellum in these specimens varies in length from 1μ to 4μ , which agrees very closely with the estimate made by BRUCE *et al.* (1912), whereas the measurements of some of the other authors, especially those of WALRAVENS (1926), are exaggerated, probably for the reason indicated in the preceding section (cf. Table I).

With the view of estimating the proportion of forms with and without a free flagellum, a count was made of one thousand individuals in each of three strains of *T. simiae*, the result of which is shown in Table III, in which the trypanosomes are divided into three groups: (1) those in which a free flagellum is definitely absent, (2) those in which a free flagellum is only apparent, the accompanying undulating membrane probably being obscured for reasons given above, and (3) those in which a free flagellum is distinctly visible.

TABLE III.

DISTRIBUTION OF FORMS IN *Trypanosoma simiae* WITH RESPECT TO THE FREE FLAGELLUM.

Strains (Belgian Congo).	Percentage of individuals in which a "free flagellum" is as follows:—			No. of individuals counted.
	Absent.	Apparent.	Present.	
Dr. BOURGUIGNON's "10F"	82.2	13.9	3.9	1,000
Dr. SCHWETZ's "CM"	82.4	14.9	2.7	1,000
" " "C477"	89.8	9.0	1.2	1,000

From Table III it is seen that in over 80 per cent. of individuals in *T. simiae* a free flagellum is definitely absent, while in up to 3.9 per cent. of individuals it is present. As regards the intermediate group ("Apparent"), comprising

up to 14.9 per cent. of individuals examined, its authenticity is admittedly uncertain, in that its interpretation is largely a matter of individual judgment. If examined uncritically these forms might be regarded as having a true free flagellum and be assigned to the fourth column. It should be added that in imperfect preparations the terminal portion of the undulating membrane fades away entirely, and in such films not only the forms in which a free flagellum is "apparent," but many of those in which it is definitely absent, could be interpreted as possessing a true free flagellum. In fact, this is what actually happened in the case of BRUCE's film of *T. simiae* examined by me. Both its present appearance and the published figures of the trypanosome (BRUCE *et al.*, 1912) leave little doubt as to the original excellence of the preparation. However, some parts of the film have deteriorated with time and use, in consequence of which it now shows a larger proportion of trypanosomes with an "apparent" free flagellum than was indicated by BRUCE. It is inconceivable that these would have escaped his notice had they been present in the film in 1912. Personally, for reasons given above, I am inclined to regard the forms with an "apparent" free flagellum as belonging to the typical group in which the undulating membrane extends to the tip of the flagellum.

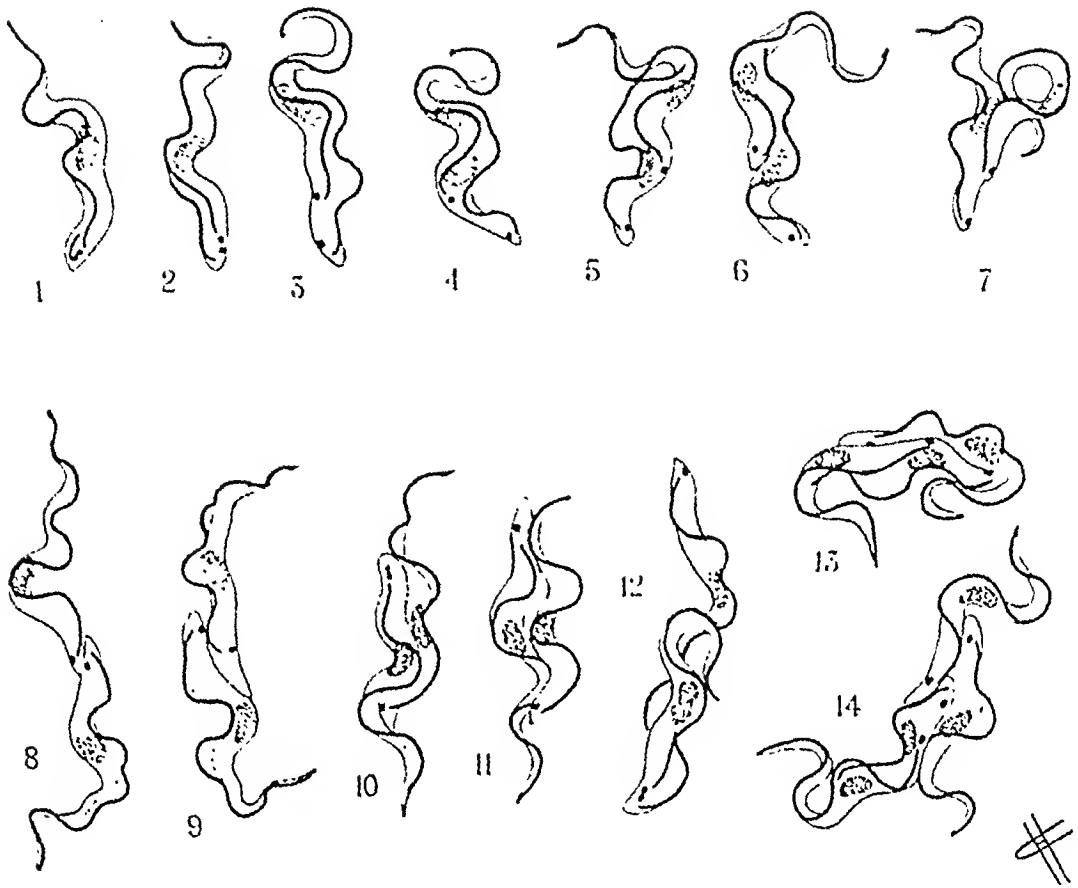
4.—AUTO-AGGLUTINATION IN *T. simiae*.

In their account of *T. simiae*, BRUCE *et al.* (1912) describe certain appearances as a unique and—it might be added—unaccountable form of division, "in which the trypanosomes appear to slip past one another until they are joined by their non-flagellar ends." (The figures illustrating this process show pairs of trypanosomes in a "head-to-tail" arrangement, similar to those depicted here in Fig. B, 8-10). These authors also noted clumps of trypanosomes (like those in Fig. B, 13, 14) which they attributed to multiplication which "took place so rapidly that the individual trypanosomes had not time to disengage themselves."

Though none of the observers whose films I have examined refer to this phenomenon, the forms represented in Fig. B (8-14) were found in abundance in all the strains of the pig-trypanosome examined by me. In addition to these forms there were present others, exhibiting all stages of division of the conventional type (Fig. B, 1-7). This finding confirmed my first impression that the process described by BRUCE and his collaborators did not represent division, but some other phenomenon.

The clue to its nature was provided by the blood of mice infected with *T. congolense*, stained films of which showed the same "head-to-tail" arrangement of trypanosomes in pairs as in *T. simiae*.

This appearance is due to the auto-agglutination or agglomeration of the trypanosomes, which can readily be observed microscopically in fresh preparations of blood containing *T. congolense*. In these the trypanosomes show a marked tendency to adhere to one another. As soon as two or more individuals collide they become attached at the points of contact, after which they coil and twist

FIG. B. *Trypanosoma simiae*.1 to 7 : *Stages of division.*

1—kinetoplast dividing, with new flagellum arising from its posterior half ; 2—kinetoplast divided into two ; new flagellum growing along edge of the body ; 3—continuation of the process : body being drawn out posteriorly, kinetoplasts widely separated ; 4, 5—later stages : division of nucleus and further separation of kinetoplasts ; 6—division completed : daughter—individuals well differentiated ; 7—daughter—individuals about to separate from each other.

8 to 14 : *Auto-agglutination.*

8—first contact established ; 9, 10, 11—agglutinated pairs (" head-to-tail " arrangement) ; 12—separation of agglutinated individuals (in the lower one division has commenced) ; 13—agglutination of dividing and non-dividing individuals ; 14—agglutination of a dividing individual with two non-dividing ones.

(All the figures were drawn with the aid of a camera lucida, at $\times 2,000$.)

around each other vigorously in an apparent effort to disengage themselves. When the union involves only two trypanosomes—as is most usually the case—the mutual arrangement of the two partners varies considerably, but it was observed that the maximum degree of adhesion resulted when the two individuals adopted the “head-to-tail” disposition. When orientated in this manner, they remained attached for longer periods of time than in any other position. Such pairs could frequently be seen disengaging themselves by the two partners sliding apart in a backward direction, *i.e.* with the posterior ends of the body leading. Owing to the relative stability of such couples they are the ones most commonly encountered in stained preparations. Their appearance in *T. congolense* is similar to that in *T. simiae*—represented in Fig. B, 8–14—leaving no doubt that the latter are due to the same phenomenon. As already stated, the union of the trypanosomes may occur at any point of the body, and the sequence from 8 to 12, in Fig. B, is only a particular example of a case when contact is first established by the posterior ends, the partners subsequently sliding apart as described above.

As far as could be ascertained from the literature and from direct observations, this type of agglutination does not commonly occur in any other trypanosome, but *T. congolense* and *T. simiae*. It was first noted in another member of the *congolense*-group, *T. dimorphon*, by LAVERAN and MESNIL (1904). As will be shown below, the fact that agglutination in couples disposed “head-to-tail” is a peculiar characteristic of trypanosomes of the *congolense* group can be used as an aid in the diagnosis of infections with these trypanosomes. Finally, lest there might be any temptation to interpret the phenomenon described above as a sexual process, it should be emphasized that in no case have any nuclear changes been observed in the agglutinating flagellates and, moreover, the union may take place indiscriminately between two non-dividing forms (Fig. B, 8), between these and dividing forms (Fig. B, 10, 12, 13), between dividing forms, and between more than two individuals (Fig. B, 14).

5.—DIVISION IN *T. simiae*.

It has been demonstrated in the previous section, that the description of division in *T. simiae* given by BRUCE and his collaborators was based on a misinterpretation of the process of auto-agglutination.

I have observed numerous forms of *T. simiae* in various stages of division, which is of the conventional type of equal binary fission, but with certain peculiarities not previously noted. The process begins with the division of the kinetonucleus in the direction of the long axis of the body; this is followed by the growth of a new flagellum which invariably arises from the posterior daughter-kinetonucleus (Fig. B, 1, 2). As division proceeds the distance between the daughter-kinetonuclei increases progressively, while the new flagellum grows in length and runs along the outer margin of the body, exteriorly to the old flagellum. In the meantime, the nucleus divides, its daughter elements separating widely

from each other (Fig. B, 3, 4). At this stage the daughter-individuals become fully formed and differentiated from each other, the couple having a linear arrangement with one of the partners overlapping the other (Fig. B, 6). As is usual in trypanosomes, the separation of the two flagellates takes place by fission of the cytoplasm commencing from the anterior end (Fig. B, 7). An identical type of division was observed by me in *T. congolense*.

In a previous paper (HOARE, 1936) I have indicated that the method of reproduction affords a means of differentiating some of the species of the *levisi*-group of trypanosomes. Since the type of division in *T. simiae* and in *T. congolense* described above appears to be peculiar to the *congolense*-group, it can likewise be employed for diagnostic purposes. Whereas in members of the *brucei*- and *evansi*-groups the products of division remain united by their posterior extremities till the end of the process, giving the couple a bifid appearance, in the *congolense*-group the dividing form has a stepped appearance.

As regards the third group of pathogenic trypanosomes, *viz.* the *vivax*-group, the type of division seen by me in *T. vivax* appears to occupy an intermediate position between the bifid and the stepped types.

IV.—OBSCURE OUTBREAKS ATTRIBUTABLE TO *T. simiae*.

In addition to the cases of acute porcine trypanosomiasis in which it was possible to demonstrate by direct examination of blood films that the causative agent was *T. simiae* (cf. Section III), there remain to be considered a number of epizootic outbreaks (described in Section II) in which the clinical manifestations were the same as in those of undoubted *T. simiae* infections, but which were attributed to various other named and unnamed trypanosomes. Though films are not available for the re-examination of these, when the published data regarding these parasites are scrutinized in the light of our present knowledge of *T. simiae*, they reveal many points of resemblance to this species, as will be seen from the critical analysis that follows.

The trypanosome described by OCHMANN (1905) from the earliest known outbreak in German East Africa was said to be shorter and thicker than *T. brucei*, and was therefore provisionally named *T. suis*. This difference is brought out in the photomicrographs accompanying the paper and was noted already by LAVERAN and MESNIL (1912). The two trypanosomes depicted in the paper, however, bear some resemblance to *T. simiae*. MAYER (1912), who appears to have seen both OCHMANN's trypanosome and the one found by GEISLER (1912) in the East African warthog, identified the latter as *T. suis*. This trypanosome is described as being broad and stumpy and possessing a free flagellum. In the figures given by MAYER it is similar in appearance to the predominant form of *T. simiae*. In view of these facts it is highly probable that the trypanosomes observed by OCHMANN and by GEISLER were actually *T. simiae*.

In his report on the outbreaks in Southern Rhodesia BEVAN (1917) compares the trypanosome found in the pigs to *T. pecorum* (= *T. congolense*) from which

it differs "notably in the fact that the body appeared more flexible and the undulating membrane more highly festooned" and adds that the two were readily distinguishable. The only trypanosome which conforms to this description is the classical *T. simiae* and there can be little doubt that this species was responsible for the epizootic.

We now turn to the outbreak described by ARMFIELD (1922) from Tanganyika Territory. In this case the trypanosomes were identified by HORNBY (1923) first as *T. vivax* and subsequently as *T. uniforme*. HORNBY noted two peculiarities in this trypanosome, hitherto not recorded for members of the *vivax*-group: first, its high virulence for pigs, and secondly the occurrence of "multiple fission." HORNBY's illustrations of *T. uniforme* at first glance appear to support his diagnosis, but a closer scrutiny of the figures shows that he was dealing with a mixed infection: while his figs. 1, 2, 3, 6 and 7 actually represent *T. uniforme*, figs. 4 and 5 exhibit the marginal kinetonucleus, well developed undulating membrane and apparent free flagellum characteristic of *T. simiae*. The identification of *T. simiae* is furthermore confirmed by the occurrence of the peculiar "head-to-tail" agglutinated couples depicted in HORNBY's figs. 11 and 12, the one in fig. 12 being attached to what appears to be a dividing form of *T. uniforme*. These are the forms which HORNBY mistook for an "unusual type of fission" (fig. 11) and "multiple fission" (fig. 12). It is thus obvious that the fatal cases of pig-trypanosomiasis in this outbreak were also due to *T. simiae*, and not to *T. uniforme*. It has already been demonstrated (cf. p. 625 *et seq.*) that an earlier epizootic among pigs observed by Dr. HALL in the same colony, was due to *T. simiae* and not to *T. uniforme*, to which it was first attributed. These two species were similarly confused in the case of WALRAVENS' trypanosome (HORNBY, 1926).

In the only outbreak reported from West Africa by ALDIGÉ (1920) the exact systematic position of the causative agent was left open. The trypanosome, said to be like *T. brucei* in some respects, measured from 12μ to 25μ , and had a free flagellum varying considerably in length. There was also a marked degree of auto-agglutination of the trypanosomes in the course of which "deux ou plusieurs d'entre eux sont accolés latéralement sur une certaine longueur." If it is taken into consideration that the dimensions of these trypanosomes fall within the range of *T. simiae*, that the apparent presence or absence of a free flagellum in this species is largely a question of technique, and that the trypanosomes agglutinated in the fashion characteristic of *T. simiae*, it seems probable that the parasite seen by ALDIGÉ actually belonged to this species. As regards its likeness to *T. brucei*, one may recall the apt description of *T. simiae* given by MESNIL (1913): "l'aspect général est celui d'un *brucei* réduit de $1/4$ environ."

There remain to be considered the epizootics described by LICHTENHELD (1912) from German East Africa. These were attributed to nagana, but the evidence for this view appears to be merely presumptive. On the other hand, the fact that bovines and equines kept in proximity to the pigs escaped infection,

as well as the symptoms of the disease in pigs, all point to *T. simiae* as the probable cause of these outbreaks.

In concluding this section it can be stated that in all the outbreaks of acute porcine trypanosomiasis on record there is good reason to believe that the causative organism is *T. simiae*. In some cases (those communicated by HALL, and those described by WALRAVENS, 1926; WALRAVENS *et al.*, 1927; SCHWETZ, 1930, 1934; BOURGUIGNON and JUSSANT, 1934) this has been established directly by the examination of the original films; in other cases (described by OCHMANN, 1905; BEVAN, 1917; ALDIGÉ, 1920; HORNBY, 1923) it has been possible to identify the parasite with some degree of accuracy from the descriptions and figures, while in one instance (described by LICHTENHELD, 1920) the diagnosis is based on circumstantial evidence alone. The case for attributing all the outbreaks to *T. simiae* receives notable support from the fact—already mentioned above—that in all these epizootics the clinical picture has been identical. Finally, there is a remarkable degree of similarity in the conditions under which the infection appears to have been propagated in these outbreaks.

The reason why the majority of observers have failed to recognize *T. simiae* in the cases described by them is not far to seek. Except in the rare sporadic outbreaks amongst pigs, this trypanosome is apparently never encountered naturally in domestic animals; consequently hardly anyone has first hand acquaintance with this species.

V.—EPIDEMIOLOGY OF PIG-TRYPANOSOMIASIS.

In his review of the trypanosomiasis affecting domestic animals in Central Africa, HORNBY (1919) dismisses *T. simiae* on the grounds that "its ravages are, in economic importance, not to be compared with those of the three which we are about to study [*T. brucei*, *T. congolense*, *T. vivax*]." As a matter of fact, there could be no question of "ravages" due to *T. simiae* at all, since at that time no natural infections in domestic animals had ever been attributed to *T. simiae*, the effect of which upon these animals was known only from experimental infections (cf. p. 630).

Now, however, the position has changed, for pig-trypanosomiasis due to *T. simiae*, as described in Section II, must be regarded as of considerable economic importance to pig-breeders in tropical Africa, especially in view of the fulminating character of the disease and of our ignorance of its epidemiology, knowledge of which is limited to a few facts regarding the geographical distribution of *T. simiae*, the reservoir of infection, and the method of its transmission.

1.—GEOGRAPHICAL DISTRIBUTION.

Up to the present, infections due to *T. simiae* have been reported from the following localities (indicated by black circles in the Map, p. 623):—

(a) French Guinea (ALDIGÉ, 1920); (b) Belgian Congo (WALRAVENS, 1924-1927; SCHWETZ, 1930-1934; BOURGUIGNON, 1933-1935); (c) Tanganyika

Territory (OCHMANN, 1905 ; LICHTENHELD, 1912 ; HALL, 1920 ; ARMFIELD, 1922) ; (d) Nyasaland (BRUCE *et al.*, 1912) ; (e) Northern Rhodesia (KINGHORN and YORKE, 1912) ; (f) Southern Rhodesia (BEVAN, 1917).

There can be little doubt that the actual distribution of *T. simiae* is much wider but, owing to its relative harmlessness to most animals, its occurrence is only revealed in the presence of the susceptible host, the domestic pig, which is not extensively bred in tropical Africa.

2.—RESERVOIR OF INFECTION.

Working in Nyasaland, BRUCE *et al.* (1913*b*) made an extensive survey of the local wild animals with the object of establishing the incidence of trypanosomes in them. Out of 180 animals examined the warthog (*Phacochoerus aethiopicus*) proved to be the only one which harboured *T. simiae*. A year earlier GEISLER (1912) recorded a trypanosome from a warthog obtained in German East Africa. This trypanosome was identified by MAYER (1912) as *T. suis*, which, as was shown above (p. 637), most probably represents *T. simiae*. BOURGUIGNON (1935) also notes that warthogs occur in Katanga, from where his and WALRAVENS' cases of *T. simiae* came.

There is thus a certain amount of evidence pointing to the warthog as the natural host of *T. simiae* and the reservoir from which infection spreads to the domestic pig.

3.—TRANSMISSION.

The only definite data on the transmission of *T. simiae* were those supplied by BRUCE *et al.* (1912) and KINGHORN and YORKE (1912) whose strains of this trypanosome actually originated from animals infected in the laboratory through the bite of "wild" *Glossina morsitans*. In a later paper BRUCE *et al.* (1913*a*) described the complete cycle of development of this trypanosome which commences in the alimentary canal of the tsetse fly and is completed in the proboscis, as in the case of *T. congolense*. BRUCE *et al.* (1913) note that in the area in which their work was conducted both warthog and infected *G. morsitans* occurred in large numbers. There can, therefore, be little doubt that the initial infection in the domestic pig is acquired from the warthog through the agency of tsetse flies.

The case, however, becomes more complicated if we turn to the transmission of the infection during the epizootics of pig-trypanosomiasis. The information available is very scanty, but remarkably uniform. All the observers who have anything to say regarding the propagation of the disease emphasize the paucity or apparent absence of tsetse flies in the immediate neighbourhood in which the outbreaks took place, although these insects were usually prevalent in the

surrounding country. The following are the species noted in the localities where the outbreaks occurred: *G. palpalis*, *G. fusca*, *G. tabaniformis* and *G. brevipalpis* near Stanleyville, Belgian Congo (SCHWETZ, 1930; BOURGUIGNON, 1935); *G. morsitans* and *G. brevipalpis* in the region of Elisabethville, Belgian Congo (BOURGUIGNON and JUSSIANT, 1934); in the Tanganyika Territory Dr. HALL* found *G. morsitans* and *G. pallidipes* heavily infected, while LICHTENHELD (1912) also refers to the presence of the last-named species; *G. morsitans* was numerous in Southern Rhodesia (BEVAN, 1917).

On the other hand, in the majority of outbreaks large numbers of other blood-sucking flies, such as *Stomoxys* and Tabanidae (especially *Haematopota*), were found in immediate contact with the pigs, and there is a general consensus of opinion among the observers that these flies, and not the tsetse, were mainly concerned in the spread of the infection, though BEVAN (1917) notes that the disease was not contracted by this means in his laboratory, in spite of the fact that "the sick and healthy pigs were closely stied together and continually pestered by swarms of stomoxys which were noticed to pass from one to the other."

On the whole, there appear to be good reasons for believing that the outbreaks of acute porcine trypanosomiasis are in the first instance introduced from outside by a few tsetse flies, after which the infection is picked up by other blood-sucking flies, usually present in large numbers, and spread by them mechanically throughout the herd. BEVAN's observations would suggest that, as in the case of surra (cf. NIESCHULZ, 1930), the infection is spread by Tabanidae rather than by *Stomoxys*. However, this and other questions concerning the epidemiology of the disease stand in need of further investigations.

VI.—CLASSIFICATION OF *T. simiae*.

The present study of *T. simiae*, based on material obtained from infected pigs and upon a re-examination of BRUCE's para-type-specimens of this species, has revealed a number of important new characters which have led to an amplification and revision of the original description of this trypanosome given by BRUCE *et al.* (1912).

The structure, bionomics and nomenclature of *T. simiae*, as established at present, are brought together below.

* Personal communication.

1.—REVISED DIAGNOSIS.

Nomenclature :

Trypanosoma simiae Bruce *et al.*, 1912.

Synonyms : *T. ignotum* Kinghorn and Yorke, 1912.

Duttonella simiae (Chalmers, 1918).

T. rodhaini Walravens, 1924.

T. porci Schwetz, 1932.

[*T. congolense porci*] Schwetz, 1934.

T. suis Ochmann, 1905 ; Mayer, 1912.

Morphology in Vertebrate Host.—Polymorphic trypanosome, comprising (1) elongated stout forms with well-developed undulating membrane (ca. 90 per cent.), (2) elongated slender forms with slight undulating membrane (ca. 7 per cent.), and (3) short (*congolense*-like) forms (ca. 3 per cent.). The great majority of trypanosomes have no free flagellum : it is probably present in 1.2 to 3.9 per cent. (only in elongated forms). Kinetonucleus typically subterminal and marginal. Dimensions : total length (body + flagellum) from 12μ to 24μ , mean length from 17μ to 18.2μ . Binary fission markedly asymmetrical ("stepped"). (Note : characteristic "head-to-tail" agglutination in pairs).

Biological Characteristics.—Intermediate hosts : tsetse flies (*Glossina morsitans* and possibly other spp.) : development in intestine and proboscis (metacyclic trypanosomes). Pathogenicity : highly virulent to pigs and sometimes to monkeys ; slightly virulent to goats and sheep ; not infective to cattle, antelope, dogs and laboratory rodents.

Habitat.—Vertebrate hosts : warthog (*Phacochoerus aethiopicus*) (reservoir) and domestic pig (*Sus scrofa*) (Suidae).

Geographical Distribution : Tropical Africa, mainly Central and Eastern.

2.—SYSTEMATIC POSITION.

According to BRUCE *et al.* (1913), "*T. simiae* belongs to the same group as *T. pecorum*" (= *T. congolense*) : their general appearance is said to be similar and "it is often difficult or impossible to distinguish between a short individual of the former species and a long one of the latter." Furthermore, the two species proved to have an identical method of development in the invertebrate host (BRUCE *et al.*, 1913a). HORNBY (1919), however, suggested that *T. simiae* was "only a variety of *T. congolense* modified by passage through the warthog," but it is not clear whether this author implied that these two species were mutually convertible or whether he merely referred to the origin of *T. simiae*, as an established variety, from *T. congolense*.

The question of the exact rank to be given to *T. simiae* (specific or varietal) is, of course, a matter of convention. The important fact to be emphasized is

that this species differs from *T. congolense* in a number of essential morphological characters, such as its dimensions, its distinct polymorphism, the occurrence of forms with a free flagellum, and in the structure of its elongated forms. The only form of *T. simiae* which is indistinguishable from *T. congolense* is the short type of trypanosome (cf. Fig. A, 8, 15, 24, 28). On the other hand, I have never found in *T. simiae* the short stumpy type of trypanosome frequently seen in *T. congolense* (cf. "*T. nanum*": BRUCE *et al.*, 1911), which actually represents a replica in miniature of some of the "stumpy" forms occurring in trypanosomes of the *brucei*-group (cf. "*T. nigeriense*": MACFIE, 1913).

Since *T. simiae* possesses a number of well defined characters by which it can be differentiated from other trypanosomes, there is every reason for recognizing it as an independent species, especially in view of the fact that in many cases trypanosomes differing but slightly or indistinguishable from each other have been given specific rank.

The presence in *T. simiae* of forms indistinguishable from *T. congolense*, the absence of a free flagellum in the great majority of individuals, and the type of development in the tsetse fly, are all attributes of the *congolense*-group. On the other hand, this trypanosome would appear to have definite affinities with the *brucei*-group: like members of the latter it is polymorphic; the typical stout elongated forms of *T. simiae* and particularly those with a free flagellum (cf. Fig. A, 4, 5, 13, 19, 20) are similar to the "intermediate" forms of trypanosomes of the *brucei*-group, both as regards their general appearance and size; and, moreover, the slender "*rodhaini*"-like forms of *T. simiae* also occur in members of the *brucei*-group.

It would thus seem that morphologically *T. simiae* represents a connecting link between the *congolense*-group and the *brucei*-group, while biologically (type of development in the insect host) it belongs entirely to the *congolense*-group.

In a previous publication (HOARE and COUTELEN, 1933) an attempt was made to rationalize the classification of the mammalian trypanosomes by arranging them on a phylogenetic basis, and combining the classification with a key for their identification by the morphological characters. The present study has provided new facts in support of our views on the evolution of the trypanosomes in that *T. simiae* fills the gap between the *congolense*- and the *brucei*-groups. However, the key which conformed to the data available at that time now stands in need of readjustments to permit the inclusion of *T. simiae* with its mixed characters. I propose to deal with this question in a future publication.

VII.—SUMMARY.

This paper deals with the aetiology of an acute form of trypanosomiasis among pigs in tropical Africa.

The study of the parasites from a number of cases showed that they all represent a polymorphic trypanosome of the same type.

A re-examination of BRUCE's original *Trypanosoma simiae* revealed in it a similar polymorphism and established its identity with the trypanosome responsible for the outbreaks among pigs.

A revised description of *T. simiae* (summarized in the Diagnosis on p. 642), is given and its systematic position is discussed. It is shown that *T. simiae* has affinities both with members of the *congolense*-group and with those of the *brucei*-group, and should occupy an intermediate position.

The division and auto-agglutination in this trypanosome are described for the first time.

An account is given of all the known instances of acute pig-trypanosomiasis. While in some of these it was possible to demonstrate the presence of *T. simiae* by examination of films, in others the presence of this trypanosome could be inferred from a critical analysis of the records.

The epidemiological data concerning these outbreaks are also briefly discussed.

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A CLINICAL COMPARISON OF ATEBRIN-MUSONATE WITH QUININE BIHYDROCHLORIDE.

(A PRELIMINARY REPORT BASED ON THE TREATMENT OF 286 CASES OF
ACUTE MALARIA.)

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FOREWORD.

Atebrin-musonate, one of the latest additions to the growing list of synthetic anti-malarial drugs, is the dimethane sulphonate of atebrin. The more familiar "atebrin" of commerce is the dihydrochloride.

Unlike atebrin, atebrin-musonate is readily soluble in water. The drug was prepared by Messrs Bayer as a form of atebrin suitable for administration by intramuscular or intravenous injection. It is supplied as a yellow powder in dry ampoules containing 0.375 gramme—the equivalent of 0.3 gramme of atebrin dihydrochloride†—a quantity which is readily soluble in 2 c.c. of water.

According to instructions issued by the manufacturers the drug is best administered by intramuscular injection, though it may also be given intravenously. The intramuscular dosage suggested is 0.3 gramme on each of the two successive days. For intravenous administration the maximum single dose should not exceed 0.1 gramme, repeated if necessary two or three times within the 24 hours.

It is suggested that two intramuscular injections, each of 0.3 gramme, on successive days are generally sufficient to effect permanent cure of acute malarial infections, a view which is supported to some extent by results reported from Ceylon and Singapore.

BLAZE and SIMEONS (1935), for instance, treated a small series of twenty-one cases of acute malaria with atebrin-musonate in the Colombo General Hospital, and found that two injections of 0.3 gramme given intramuscularly on successive days were sufficient to control fever within 48 hours and to effect the disappearance from the peripheral blood of all forms of *P. vivax*, and of the trophozoites of *P. falciparum*, within four days. In a few cases there was a reappearance of parasites but in no case was there any return of fever during the period of observation.

* The malaria wards of the General Hospital, Kuala Lumpur, are under the general direction of Dr. J. W. WINCHESTER to whom we are indebted for the freedom of access to clinical material which we were afforded.

Supplies of atebrin-musonate were furnished by the local agents of Messrs. Bayer through their scientific representative, Dr. PETERS, to whom we extend our thanks.

Finally we wish to express our appreciation of the helpful advice and criticism we have received from Dr. A. NEAVE KINGSBURY, the Director of the Institute for Medical Research, Federated Malay States.

†In the following pages the dosage of atebrin-musonate will be expressed in terms of the content of atebrin dihydrochloride.

More recently VARDY (1935) has treated fifty cases of acute malaria in Singapore, and reported similar results. From a study of VARDY's detailed case records it is evident that, in most instances, two intramuscular injections of 0.3 gramme on successive days effected a rapid disappearance of parasites from the peripheral blood and a still more rapid remission of fever. VARDY refrains from drawing sweeping conclusions from the evidence at his disposal but advances the view that in atebirin-musonate "we obviously have a very powerful therapeutic agent in the treatment of malaria."

Subsequent reports from Ceylon have been somewhat less favourable. The powerful therapeutic activity of atebirin-musonate in the immediate treatment of acute infections has been generally confirmed but attention has been drawn to the occasional occurrence, particularly among children, of serious symptoms which are believed to be related to the administration of the drug, while doubt has been thrown on the permanency of cure in a relatively high proportion of the cases treated (BRIERCLIFFE, 1935).

PRESENT INVESTIGATION.

In May of the present year a generous supply of atebirin-musonate was placed at the disposal of the Malaria Research Division of the Institute for Medical Research, Kuala Lumpur, by the local agents of Messrs. Bayer, and in June an experimental comparison of this drug with quinine bihydrochloride for the treatment of acute malaria was commenced in the malaria wards of the General Hospital adjoining the Institute.

Between June and September 286 cases of acute malaria were treated by the writers either with atebirin-musonate or with quinine bihydrochloride for the purposes of control. The present report is an account of the results obtained to date. The enquiry is being continued.

Type of Case.

The subjects of this enquiry are almost entirely of Malay, Chinese, or Indian race. The former were drawn from the peasantry of the neighbouring villages, while the Chinese and Indians were generally labourers from local rubber estates and tin mines.

Most of the patients were drawn from districts where malaria smoulders throughout the year and for this reason primary infections were in a minority, but in view of the difficulty of obtaining precise medical histories from Asiatic labourers the proportion of primary to recurrent infections was not determined.

Table I indicates the distribution of the three types of malaria in the atebirin-musonate and quinine case series.

TABLE I.

		Subtertian.	Benign tertian.	Quartan.
Atebrin-musonate series	..	117*	40	7
Quinine series	..	89	29†	0

* This figure includes twenty cases treated intravenously with small doses of atebirin-musonate and not controlled by the treatment of parallel cases with quinine.

† At one stage of the enquiry, cases of benign tertian malaria were relatively few, and quinine controls were temporarily suspended in order to facilitate the rapid collection of data regarding atebirin-musonate.

Methods of Enquiry.

Available cases of acute uncomplicated malaria admitted to the malaria wards during the period of the investigation were treated in alternating sequence with atebirin-musonate or with quinine bihydrochloride, irrespective of sex, race, or duration of infection.

Cases falling within the following groups were excluded for reasons which are sufficiently obvious.

(a) All afebrile cases.

(b) All cases of low grade parasitization (initial trophozoite counts of less than 500 per c.mm. in the case of subtertian infections, and 200 per c.mm. in benign tertian and quartan infections).

(c) All cases with a history of previous malarial treatment within 3 days of coming under experimental observation.

(d) All mixed infections.

(e) All children under 12 years.

All cases were under full hospital observation and control. Temperatures were recorded four times a day. Thick blood films, stained by the Giemsa technique described by GREEN (1931) were examined daily during an observation period of 7 days and parasites were counted daily by SINTON's fowl corpuscle method.

The general aim of the enquiry was to compare atebirin-musonate treatment with standard quinine treatment and not two injections of atebirin-musonate with two injections of quinine bihydrochloride. Two injections of quinine bihydrochloride cannot be regarded as an efficient course of treatment for acute malarial infections and a comparison of the two drugs under strictly parallel conditions would of necessity have little more than an academic significance.

Quinine bihydrochloride was administered orally in solution twice a day for 7 days at a dosage calculation according to body weight.

Atebrin-musonate was given by intramuscular or intravenous injection. For intramuscular administration the contents of one ampoule were dissolved in approximately 2.5 c.c. of cold sterile tap water. Solution was usually complete within 2 or 3 minutes.* For intravenous use 0.3 gramme of the drug was dissolved in 9 c.c. of water. The total intramuscular dosage was either 0.6 gramme or 0.9 gramme: the total intravenous dosage varied from 0.2 gramme to 0.6 gramme.

* The manufacturers recommend that 0.3 gramme, the contents of one ampoule, should be dissolved in 9 to 10 c.c. of water as stronger solutions may give rise to tissue irritation. We have had no evidence that the solutions of over three times this strength used by us have been unduly irritative while on the other hand we regard 10 c.c. as somewhat too large a volume for routine intramuscular injection.

Quartan malaria is relatively uncommon in Kuala Lumpur, and during that period of the enquiry seven cases only were available for treatment. These cases each received intramuscular injections of 0.3 gramme of atebirin-musonate on two successive days. Trophozoites and schizonts were absent from thick films taken from the peripheral blood within an average period just under 4 days, while the duration of fever averaged approximately $1\frac{1}{2}$ days.

Effect of Atebrin-musonate on Gametocytes.

It is well recognized that atebirin is of limited value as a gametocide for it has little effect on the numbers or on the viability of the gametocytes of *P. falciparum*, and it is, perhaps, only to be expected that atebirin-musonate, a salt of atebirin which, presumably, owes its plasmocidal activity to its atebirin content, should exhibit similar effects.

Gametocyte counts were made daily on all our patients. Table II summarizes the day by day gametocyte incidence. It will be noted that benign tertian gametocytes rapidly disappeared during atebirin-musonate treatment but that subtertian gametocytes, on the contrary, were present in a high proportion of cases on the 7th day.

TABLE II.
NUMBER OF GAMETOCYTE CARRIERS FROM DAY TO DAY DURING TREATMENT.

	1st Day.	2nd Day.	3rd Day.	4th Day.	5th Day.	6th Day.	7th Day.
AM/ST. (94 cases)	26	36	41	49	56	58	52
Q/ST. (88 ")	19	31	38	49	59	56	51
AM/BT. (40 ")	28	26	16	3	0	0	0
Q/BT. (29 ")	18	18	14	6	1	1	1

The viability of subtertian gametocytes was apparently little affected by the presence of atebirin-musonate in the system for five out of six crescent-carriers on whom mosquitoes were fed at varying intervals from the 4th to the 10th day after the commencement of treatment were found to be infective.

Deaths.

There were admitted 24 hours after onset of quinine treatment.

AM, condition. Died within 3rd day.

of a total of 288 infections and received atebirin-musonate.

WS :—

rs. S.T. ring Oedema of

.T. ring

All four cases died within 24 hours of treatment and one

mm. General condition. Dyspnoeic.

. Died on

AM, ST. 35. Male Chinese aged 21 years. S.T. rings too numerous to count in thick films. Thin film count showed 31 per cent. of R.B.C's infected. Died within 24 hours.

Q, ST. 68. Male Chinese aged 33 years. S.T. rings 180,000 per c.mm. Died within 24 hours.

It is perhaps not entirely fair to include Cases 5 and 35 in the list. The infections from which these patients were suffering were of such overwhelming intensity that recovery under any treatment was unlikely, and it was entirely by coincidence that they fell to the atebirin-musonate series.

Relapses.

The minimum period of observation in hospital was 7 days, and although a few individuals were induced to stay as long as 3 or 4 weeks, it was found that after a week most patients clamoured for discharge to return to the districts where they had originally become infected.

Under such circumstance it was manifestly impossible to obtain precise data of relapse rates.

It was found, however, that a number of patients returned to hospital with the same type of malaria within a few weeks of discharge and it is probable that most of these infections were true relapses.

These recurrent infections are analysed in Figure 5. It is not suggested

FIGURE 5.
APPARENT RECURRENCES OF INFECTION WITHIN TEN WEEKS OF TREATMENT.

Serial Number.	Dosage.		Interval in Weeks before Reappearance of Trophozoites.									
			1	2	3	4	5	6	7	8	9	10
AMST 3	0.6 gramme	1 M.			●							
" 6	0.6 "	1 M.		●								
" 10	0.6 "	1 M.			●							
" 28	0.6 "	1 M.		●								
" 31	0.6 "	1 M.	●									
" 60	0.2 "	1 V.	●									
" 79	0.9 "	1 M.									●	
" 84	0.9 "	1 M.		●								
" 90	0.9 "	1 M.								●		
" 100	0.4 "	1 V.				●						
AMBT 5	0.6-0.9 gramme	1 M.			●			●				
" 7	0.6-0.6 "	1 M.				●				●		
" 23	0.6 gramme	1 M.						●				
" 25	0.6 "	1 M.				●						
" 29	0.6 "	1 M.			●							
" 32	0.6 "	1 M.				●						
" 33	0.6 "	1 M.				●						

Quartan malaria is relatively uncommon in Kuala Lumpur, and during that period of the enquiry seven cases only were available for treatment. These cases each received intramuscular injections of 0.3 gramme of atebirin-musonate on two successive days. Trophozoites and schizonts were absent from thick films taken from the peripheral blood within an average period just under 4 days, while the duration of fever averaged approximately $1\frac{1}{2}$ days.

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Deaths.

There were four deaths out of a total of 288 cases treated. All four cases were admitted with heavy subtertian infections and three of the four died within 24 hours of admission. Three received atebirin-musonate treatment and one quinine treatment. Details are as follows :—

AM, ST. 2. Male Indian aged 32 years. S.T. rings 35,000 per c.mm. General condition poor, very anaemic (Hb. 25 per cent.). Oedema of face and abdomen. Dyspnoeic. Died within 24 hours.

AM, ST. 5. Male Chinese aged 30 years. S.T. rings 700,000 per c.mm. Died on 3rd day.

AM, ST. 35. Male Chinese aged 21 years. S.T. rings too numerous to count in thick films. Thin film count showed 31 per cent. of R.B.C's infected. Died within 24 hours.

Q, ST. 68. Male Chinese aged 33 years. S.T. rings 180,000 per c.mm. Died within 24 hours.

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The minimum period of observation in hospital was 7 days, and although a few individuals were induced to stay as long as 3 or 4 weeks, it was found that after a week most patients clamoured for discharge to return to the districts where they had originally become infected.

Under such circumstance it was manifestly impossible to obtain precise data of relapse rates.

It was found, however, that a number of patients returned to hospital with the same type of malaria within a few weeks of discharge and it is probable that most of these infections were true relapses.

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" 25	0.6 "	1 M.				●						
" 29	0.6 "	1 M.			●							
" 32	0.6 "	1 M.				●						
" 33	0.6 "	1 M.				●						

that this affords an accurate indication of the atebtrin-musonate relapse rate for it is by no means certain that every patient with a relapse returned to hospital, nor is it certain that those who did return had not acquired a fresh infection. However, the figures are presented for what they are worth.

One case treated with atebtrin-musonate (AM, ST.84) relapsed while still under observation in hospital. This case is interesting in that atebtrin was present in the system and was still being excreted in the urine in appreciable quantity when parasites and fever returned 17 days after the completion of treatment.

EXCRETION OF ATEBRIN-MUSONATE.

Atebrin may be demonstrated in the urine of all cases undergoing atebtrin-musonate treatment. During the course of the present series the urines of all atebtrin-treated cases were examined as a routine by ultra-violet light,* under which atebtrin fluoresces strongly. By using this method it is possible to demonstrate atebtrin in such minute quantities as 1 in 2,500,000. Quinine also fluoresces under ultra-violet light—a bright violet colour—but in most cases Mayer's test was considered sufficient to demonstrate its presence.

By means of a series of standards of known dilutions of atebtrin in urine, it was possible to get some idea of the amount of atebtrin being excreted. No attempt, however, was made to carry out accurate quantitative analysis and the test was not regarded as giving more than a rough idea of the amount of atebtrin present.

In the present series, all cases on full atebtrin-musonate treatment showed a well marked reaction on discharge from hospital, usually 5 to 6 days after treatment had stopped. It was, unfortunately, not possible to keep most of our patients in hospital longer than a week or 10 days but two cases remaining for a longer period were still excreting atebtrin in the urine 3 weeks after the last dose of atebtrin-musonate. It is therefore apparent that atebtrin is retained in the body for a considerable period.

Urine from several cases was examined at short intervals after administration of atebtrin-musonate, and atebtrin could usually be demonstrated in appreciable concentration between 5 and 10 minutes after injection. This was found to be the case whatever the route of injection and it does not appear, therefore, that the intravenous route has any advantage over the intramuscular route in regard to the rapidity of absorption of the drug.

As far as can be gathered from the tests applied in this series of cases, atebtrin appears in the urine a few minutes after injection of atebtrin-musonate, is excreted steadily for several days, and then slowly disappears over a period of weeks.

* The technique is as follows: 10 c.c. of urine in a test-tube is alkalisied with a few drops of a saturated solution of potassium carbonate, 0.25 c.c. of amyl alcohol added, and the tube well shaken. Any atebtrin present is extracted by the amyl alcohol which forms a layer on the surface. Where atebtrin is present in large amount this layer has a distinct yellow tinge to the naked eye. By means of ultra-violet light, the typical atebtrin fluorescence can be obtained in cases where atebtrin is present in very high dilutions.

J. W. FIELD AND J. C. NIVEN.

DOSAGE AND METHOD OF ADMINISTRATION OF ATEBRIN

In a leaflet issued by Messrs Bayer with each ampoule there are general instructions as to dosage. The intramuscular route is recommended. A total dosage of 0.6 gramme is said to be adequate treatment for the average case of acute malaria.

The above results are consistent with this claim though far the immediate cure of infection effected by two injections can be regarded as permanent.

With regard to the intravenous administration of atebtrin the recommendations of the makers are as follows:—

"For intravenous administration it is advisable not to give a dose of 0.1 gramme "atebrin" in adults. Injections should be given at intervals of 6 hours. This dose *may* be repeated several times a day (2 to 3 times a day.)

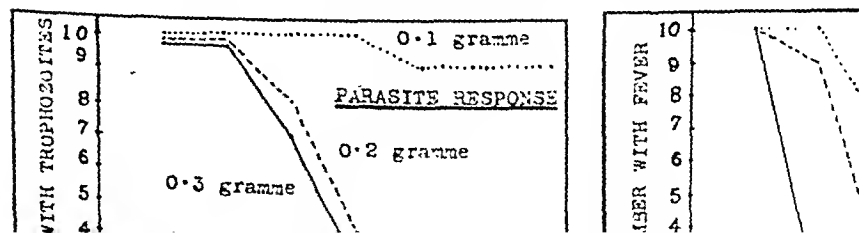
This wording is perhaps a little unfortunate as there is no indication as to whether one single injection of 0.1 gramme is not regarded as a daily dose which may at the discretion of the physician be repeated. We understand, however, from Dr. PETERS, the local scientific adviser to Messrs. Bayer, that no such implication was intended and that he does not regard a single intravenous injection of 0.1 gramme as adequate treatment for a period of 24 hours.

We had, however, before discussing the question with Dr. PETERS, made an attempt to define the minimal effective intravenous dose for the immediate treatment of acute subtertian infections with atebtrin without interest.

Three series, each of ten cases were given on each of three days intravenous injections of 0.1 gramme, 0.2 gramme and 0.3 gramme—the whole dose being given in one injection.

The response to treatment is indicated in Figs. 6 and 7.

COMPARATIVE EFFECTS OF DIFFERING INTRAVENOUS DOSES OF ATEBRIN IN 30 CASES OF ACUTE SUBTERTIAN MALARIA.



From these results it was evident that two intravenous injections of 0.1 gramme, though not entirely without effect, showed a long delayed and entirely inefficient therapeutic response. In three cases treatment at an efficient dosage became necessary before the end of the customary 7-day observation period on account of the progressive deterioration in the clinical condition.*

In the second series treated with two injections of 0.2 gramme all cases were free from trophozoites and fever within 4 days, though the response was appreciably slower than that shown in the third series receiving two injections of 0.3 gramme.

A striking feature of the treatment in the 0.3 gramme series was the rapid fall in temperature. In eight out of ten cases the temperature had fallen to normal with 24 hours, and in the remaining two cases within 72 hours.

The infections on which the curves are based showed an average range of intensity. Initial trophozoite counts per c.mm. of blood ranged from 1,000 to 212,000, with an average of 34,000 in the first series, 18,000 in the second series and 36,000 in the third series.

Despite the ready response to treatment of cases receiving adequate doses of atebirin-musonate by the intravenous route, we are in full agreement with the manufacturers that the drug is best given by intramuscular injection. Intramuscular injections of atebirin-musonate are rapidly effective and the appearance of traces of the drug in the urine within 10 minutes of an intramuscular injection of 0.3 gramme indicates a rate of absorption sufficiently rapid for even the gravest of infections.

Toxicity.

Reports have recently appeared which suggest that atebirin-musonate may not, under all circumstances, be an entirely safe drug to use. Unpleasant sequelae and even death have been attributed to the direct effects of the drug.

VARDY (1935) found that a number of his cases complained of feeling "knocked out" by the treatment. One case developed epileptiform convulsions 2 hours after the second injection of 0.3 gramme.

BRIERCLIFFE (1935) has collected and summarized the experiences of the Ceylon Medical Department during the recent malaria epidemic in which atebirin was used on a large scale. He records four instances among 681 individuals receiving injections of atebirin-musonate in hospital in which death was believed to be due to the effects of the drug. Three of these cases were children under 4 years of age. Under certain conditions—in the presence for instance of severe anaemia or of defective kidney function—he believes the use of the drug to be contra-indicated, while he sounds a note of caution against its routine use for young children.

* It was for this reason that no temperature end-point was obtainable and the full 7-day curve could not be completed.

Throughout the course of the present enquiry 421 intramuscular or intravenous injections of atebtrin-musonate were given to 176 adults. In no instance were toxic effects of any significance observed which could definitely be attributed to the drug.*

A temporary fall of blood pressure of 10 to 30 mm. of mercury has been found in those instances where readings have been taken but comparative data of the effects of atebtrin-musonate and quinine bihydrochloride on the circulatory system is incomplete.

Intramuscular injections were given into the gluteus medius. They appeared to produce remarkably little local pain or tenderness. In a few instances there was a little inflammatory induration for a day or two.

Intravenous injections, particularly those of 0.2 gramme and 0.3 gramme were usually associated with temporary giddiness and faintness, passing off within a few minutes but occasionally lasting for an hour or more.

Yellow staining of the skin such as occasionally follows the oral administration of atebtrin was not observed, though it is possible that slight degrees of staining were missed on the dark skins of our patients.

SUMMARY.

1. A comparison is made between atebtrin-musonate and quinine bihydrochloride in the treatment of acute malaria.

2. 286 cases of acute malaria due to Malayan strains of *P. falciparum*, *P. vivax*, and *P. malariae*, were treated in alternating sequence with one or other of these drugs.

3. The rates at which the atebtrin-musonate and the quinine case groups became trophozoite-free and fever-free are contrasted in a series of graphs.

* We have since observed the occurrence in two subsequent cases of epileptiform fits developing shortly after treatment.

Case AM/ST.13,135, a male Tamil of 40 years, developed a series of fits a few hours after a third injection of 0.3 gramme. Except for a little giddiness recovery was complete within 24 hours. There was no history of previous fits.

Case AM/ST.144,35, a male Tamil of 38 years, developed generalised spasms 3 hours after the third injection of 0.3 gramme. The neck, back, and limbs became rigid and the patient was found resting on the heels and shoulders though at the outset consciousness was retained. The condition became rapidly worse and within half an hour consciousness was lost and Cheyne-Stokes breathing with cyanosis developed. Recovery began within an hour of onset and on the following day the patient was relatively normal. There was no history of previous fits. The excretion of atebtrin appeared to be satisfactory. There was no evidence of liver or renal deficiency. The general condition of the patient had been poor when first he came under observation. The haemoglobin percentage was 35 and oedema of the face and feet was present.

An interesting feature of this case was the presence during the fit of a copious flow from one eye of vivid yellow tears which on test showed a strong atebtrin reaction.

It should be noted that in both instances the total dosage of the drug was 0.9 gramme, i.e. 0.3 gramme above the customary upper limit.

4. It is shown that there was a tendency for trophozoites to disappear from the peripheral blood and for temperatures to fall to normal somewhat earlier among cases treated with atebtrin-musonate.

5. No toxic effects of any importance were observed (but see footnote p. 657).

6. Evidence is recorded which suggests that the minimal effective daily dose for an adult is 0.375 gramme (= atebtrin 0.3 gramme). This dose when given either intramuscularly or intravenously on two successive days effected a rapid disappearance of parasites and fever.

7. Intramuscular administration is regarded as the method of choice.

8. It is noted that absorption of the drug from the muscles is very rapid, and that atebtrin may be demonstrated in the urine within 10 minutes of an intramuscular injection of 0.3 gramme. A method of testing for the presence of atebtrin in the urine which is sensitive to over one in a million is described.

9. It was not possible to obtain precise data regarding the permanency of cure but an analysis of cases returning to hospital within 10 weeks of discharge suggests that relapses after atebtrin-musonate treatment are probably fairly common.

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MALARIA EPIDEMICS AND SUN-SPOT CYCLES.

BY

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In his paper on the recent epidemic of malaria in Ceylon GILL* has suggested that a definite correlation exists between the maximum and minimum development of sun-spots and epidemics of malaria. It seems on examination that this correlation is not as close as GILL supposed and it may be well therefore to consider briefly what is known of sun-spots and associated phenomena.

Systematic observation of the sun with a telescope or even by the unaided eye, if sufficiently carefully pursued, will soon show anybody that from time to time dark spots appear on the bright surface.

This was noted by the Chinese as far back as the beginning of the Christian Era, and has been the object of much study by astronomers everywhere since the invention of the telescope in 1610. What is the nature of these spots?

The bulk of the sun's light is given out by a rather thin, intensely bright layer in the sun called the photosphere. This is regarded as the surface of the sun, but it can only be so conventionally as the sun is entirely composed of gas and so cannot have a sharply defined free surface. The photosphere however appears to be quite sharply defined, and what exactly it is and why it is so much brighter than the rest of the sun is not known for certain.

Sun-spots appear to be holes in the photosphere, blown in it by currents of gas rushing upwards and downwards in a peculiar manner. There is reason to suppose that the current is upwards at the edge of the spot and downwards in the middle, though this may sometimes be reversed. It is well-known, however, that the temperature of the spots is lower than that of the surrounding parts of the sun by about $1,000^{\circ}\text{C}$., the general temperature of the sun's surface being about $6,000^{\circ}\text{C}$. The expansion of upward rushing gas and the descent of cool gas accounts for this in an adequate manner. In addition to these movements the spots are the seat of a very powerful magnetic field which is sometimes positive and sometimes negative, these polarity differences following a fairly constant plan.

* GILL, C. A. (1936). Some points in the epidemiology of malaria arising out of the study of the malaria epidemic in Ceylon in 1934-35. *Trans. Roy. Soc. Trop. Med. & Hyg.*, xxix, (5), 427.

The number and area of the spots and their distribution on the sun also follow a regular rhythm. They occur in a cycle of between 11 and 12 years, increasing from a minimum when there may be no spots visible sometimes for months, increasing during 3 or 4 years to a maximum during which the sun is practically never without some conspicuous groups, then diminishing during about 6 years to the next minimum. This is not, however, an invariable cycle. Considerable extensions of the period have been recorded and during one period of 70 years, 1645 to 1715 A.D., hardly any spots were seen on the sun and sometimes none at all for periods of 10 years at a stretch.

Other changes visible in the sun are generally connected with the sun-spot cycle in their activities, but are not so definitely periodical as the spots. Elevated bright patches called faculae and eruptions, of gases and of certain metallic vapours, known as prominences, are more abundant at periods of sun-spot maximum, but are by no means absent at periods of spot minimum.

Spots are never seen within a zone of 40° from the sun's poles and almost never within 5° from the sun's equator. Prominences however occur freely at all parts of the sun's limb and are often conspicuous near the poles at times of sun-spot minima.

It sometimes happens also that powerful magnetic fields develop in the sun, similar to those associated with large spots, but with no spot associated with them.

It will thus be seen that sun-spots are only one of several manifestations of activity in the sun, which are connected with one another to a certain extent, but are not necessarily dependent on one another.

The next thing to consider is how changes in the sun may affect things on the earth.

There is no need to labour the point—how a change in the sun's heat or radiation would affect the earth. If the change is considerable it is bound to have an effect. We must therefore ask, does the sun's heat change appreciably?

A great deal of investigation has been directed towards the measurement of the sun's heat as received at the surface of the earth. This is expressed as a standard, known as the Solar Constant, which is the number of gramme-calories received from the sun per minute on a surface of 1 square cm. at right angles to the sun's rays. The average is 1.93, but when we come to consider variations, enormous difficulties are encountered, because we have to measure the heat received through the earth's atmosphere, and many variable factors combine to modify the measured constant. The density of the atmosphere, the amount of cloud, the amount of water vapour, the amount of dust all modify the Solar Constant as measured on the ground, and as these factors are different at different and often inaccessible levels, their effect on the sun's heat cannot be accurately estimated.

Estimates have been made of course, and corrections for all these and many

other factors attempted, but whether the figures obtained really approach the actual heat per square cm. received from the sun outside the earth's atmosphere, is by no means certain. Measured thus, the Solar Constant shows many variations, up to 5 per cent. of the total amount. These variations have been plotted during many years now and a great variety of periodicities have been described; thus changes occurring at intervals of 7 days, 10 days, half-year, 4-5 years, of 11-12 years and longer periods up to centuries have been suggested. It has even been argued that the well-known irregularities of "the weather" would not occur if the sun's radiation were uniform (CLAYTON, 1923).

Many causes have been suggested for the sun-spot cycle; it has been argued that Jupiter is the cause, because his period of revolution round the sun is nearly the same as the mean sun-spot cycle. Effects due to Saturn, Mercury and Venus have been described also, and it appears to be a fact that more sun-spots fade away on the side of the sun next the earth than on the other side (MAUNDER), so that the earth has some effect on them, but although outside influences may modify them, their cause is deep within the sun itself. There is strong evidence that the bigger the spot, the deeper down in the sun is its origin, but this cannot be further discussed here.

The one effect on the earth of sun-spot activity which is established fully is a magnetic effect. Outbursts of large spots near the central part of the sun's disc are so commonly followed by magnetic storms and brilliant displays (in high latitudes) of "northern lights," that it is certain that there is a connection between them. Several such have come under my own observation; but here again, many spots have no magnetic effect on the earth, and occasionally big magnetic disturbances arise from certain areas in the sun where a spot has not appeared. These magnetic disturbances are sometimes so great as to throw all the submarine telegraph cables out of action, while they last.

When we come to consider a possible connection between the sun-spot cycle and epidemics of diseases such as malaria, it is necessary to make certain preliminary assumptions.

It is not, I think, justifiable to suppose a direct effect on what GILL calls the "epidemic potential," that is, an effect on the actual virulence or disease-producing capacity of the causative organism.

It has, I know, been recently claimed in some sections of the public press, that plague, pestilences, and famine, battle, murder and sudden death are to be attributed to the malign influence of solar activity as shown by the prevalence of sun-spots. But it only needs an examination of the history of the remarkable 70-year period of solar quiescence, 1645-1715, mentioned above, to disprove any such connection. This period of the world's history was just as much disturbed by wars and tumults as any other equal period. In particular the great epidemic of plague in England, followed by the great fire of London in 1666, took place in the middle of a period of 10 years, 1661-1671, during which no spot was seen on the sun, even with the telescope.

It would require a great deal of proof before a variation in the sun's energy, requiring much study to demonstrate, could be accepted as a direct cause of variations in virulence of specific organisms.

We must therefore look for indirect causes connecting sun-spots and epidemics of malaria. Changes in rainfall would be the most likely means by which such an effect could be brought about.

If sun-spots could be shown to be connected with excessive rainfall in semi-arid countries, or with a great deficiency of rainfall in a wet climate, the connection with malaria epidemics would be intelligible.

Our knowledge of the periodicity of sun-spots dates from the work of SCHWABE in 1826, so that now for over 100 years full records of the sun's activity are available.

Records of temperature, pressure, rainfall and winds are also to be had during the same period, for a large number of stations in different parts of the world.

None of these show any very definite and obvious connection with the sun-spot cycle, as numerous other factors produce large effects, which do not seem to fit the 11-12 year period. When, however, averages are taken over the whole period, it is found that a small effect corresponding to the sun-spot period emerges.

The effect is very patchy and often of an opposite kind in places fairly close together. For example, there is an increase of frequency in cyclonic storms at sun-spot maximum in the north and in the south of the United States, but exactly the opposite in the central part of the country.

Also in the Bay of Bengal and the South Indian Ocean hurricanes appear to increase with sun-spots; in the West Indies and the South Pacific Ocean they diminish with increasing sun-spots and in the North Pacific Ocean they increase at sun-spot maximum in spring, summer and autumn, and diminish largely in the winter (HUNTINGTON, 1927).

KÖPPEN (1890) correlated 20 million observations over 100 years on world temperature and sun-spots, and found that the mean temperature of equatorial regions was 0.6° C. and of temperate regions 0.4° C. higher at sun-spot *minima* than at *maxima*. HUMPHREYS (1920) and ABBOT and FOWLE (1913) come to the same conclusion.

In the case of barometric pressure a difference has been made out amounting to not more than plus or minus one millibar ($6/100$ of an inch) at maximum of spots, compared with that at minimum, an amount which is too insignificant to produce any meteorological result in most cases. The differences in annual rainfall due to the same causes range from none up to 0.4 inches over the greater part of the world. A few small areas show a difference of 0.8 of an inch annually. The total rainfall in many of these places is of the order of magnitude of 100 inches annually and of some much more. These are general world-wide considerations, but it can be seen that they are not such as to determine

epidemics of malaria anywhere, through temperature, humidity or rainfall changes.

We must now consider the application of these phenomena to conditions in Ceylon.

Broadly speaking the weather conditions favouring the spread of malaria in Ceylon are deficiencies of rainfall in the wet zone enough to cause prolonged lowness of the water in rivers and streams, and in the dry zones abnormal heavy rain during the usually dry season, leading to an unusual number of pools of water, persisting for considerable periods.

Let us see whether this kind of weather is associated with years of sun-spot maxima and minima in Ceylon.

In the table the rainfall average for each place is given in the first column, for all the sun-spot maximum years since 1875; in the second column for all the years half way between maximum and the following minimum; in the third column for all the years of minimum; and in the fourth for the years midway between minimum and the following maximum.

The numbers in brackets show the absolute maximum and minimum rainfall for any one year, from the figures employed in the averages.

TABLE A.

FIGURES FROM COLOMBO OBSERVATORY RECORDS.

		At Sun-spot Maximum.	Half Down.	At Sun-spot Minimum.	Half Up.
Wet	Colombo (not malarious)	95 (140)	80	101	103 (71)
Wet	Kurunegala (very malarious)	80	84	92	100 (140) (67)
Dry	Anuradhapura	54 (39)	49 (75)	54	58
Very wet	Ratnapura	152 (135)	150	165 (191)	170
Dry	Puttalam	42	40 (34)	47	53 (86)
Dry	Mannar	36 (58)	39	33 (21)	44
Dry	Jaffna	50	48	46 (30)	54 (78)
	Badulla	75	65 (52)	76 (52)	89 (151)
Hill	Hakgala	90 (75)	90	105	107 (119)
Hill	N'Elia	84 (68)	92	106 (128)	97
Hill	Kandy	78 (64)	87	95 (117)	86
Dry	Trincomalie	65	58	68 (35) (95)	57
Dry	Batticaloa	64	58	67 (35) (113)	65
Dry	Hambantota	39	40	44 (60)	40 (18)
Wet	Galle	84 (68)	95	117 (143)	102

It will be seen that only in N'Eliya, Kandy and Galle is there a correspondence between maximum rainfall and sun-spot minimum and *vice versa*.

In Hambantota there is also the same correspondence but the variation is very small. Kandy and Hambantota suffered from the last malaria epidemic.

None of the eleven other stations given show any sign of sun-spot maxima and minima having any regular effect on the rainfall.

The figures are averages of all the maxima and minima since 1875.

CONCLUSION.

The relation between malaria epidemics and sun-spot activity is not as close as GILL would have us believe either in Ceylon or elsewhere. The last two outbreaks in Ceylon, 1934 and 1928, do, it is true, coincide with a minimum and a maximum of sun-spots, but from 1923 back to 1905, epidemics both in Ceylon and elsewhere, taking GILL's own dates, with one or two necessary corrections, have prevailed during one and a half complete sun-spot cycles with absolute impartiality at sun-spot maximum, minimum and every stage in between. This is shown on the chart where the curve is based on Greenwich sun-spot numbers as given by MAUNDER and NEWTON (1936) and epidemics of malaria are indicated by E.

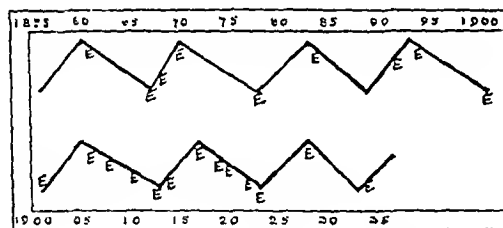


TABLE B.

SUN-SPOT CYCLE
GREENWICH NUMBERS.

E = Malaria Epidemic (GILL).

The "periodicity" of virulence of malaria parasites may be a reality, which epidemiologists are endeavouring to explain. To attribute it to the influence of a solar cycle, in the absence of convincing proof, would be as logical as attributing the length of the lunar month to the rotation of the sun upon its axis, because both take nearly exactly the same time, or to attribute the menstrual period in women to the rotation of the sun and moon because the period is nearly the same.

An indirect effect through changes in rainfall certainly cannot be maintained in Ceylon, because variation in the rainfall in most places does not follow the sun-spot cycle, and where it apparently does so the effect is too small to influence the breeding of anopheles.

Finally there is grave doubt whether the measured changes in the Solar Constant can be the cause of the weather changes attributed to it,

Changes of about 0.5° C. in the mean temperature of the world, variations of 10 mm. in the annual rainfall in various places attributed to changes in the Solar Constant, can only be very tentatively attributed to this cause so long as the errors in measurement of the Solar Constant are unknown.

There are many known causes of error, and the effect of the atmosphere with its many variables on measurements of the sun's heat, which has to come through it, cannot be exactly or perhaps even approximately eliminated, and to find a change of 1 per cent. to be due to a cause, the error of measurement of which may be 10 per cent., is a serious fallacy.

To attribute abnormal weather to sun-spot maxima or minima appears slightly unscientific at a time like the present, when very abnormal weather is occurring in Europe almost exactly half way between a minimum and a maximum.

It seems inherently improbable also that two opposite phases of the sun's activity should produce the same effect upon the incidence of malaria, unless one phase causes abnormal rain in the dry country and the other abnormal drought in the wet country, and in Ceylon the rainfall statistics give no support to this view at all. Many of the figures given for Ceylon were supplied by Dr. H. JAMESON, Superintendent of the Colombo Observatory, to whom acknowledgment is gratefully given.

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by SCHOTTE (1782) as having occurred at St. Louis de Senegal in 1778, a year in which the Gambia also was infected. SCHOTTE, though calling the disease "synochus atrabiliosa," regarded it as a severe form of malarial fever and for many years this was the generally accepted view of its nature. To determine whether a particular outbreak was yellow fever or malaria is by no means easy, more especially when it is remembered that the whole nomenclature of fevers was chaotic till well into the nineteenth century. AUGUSTIN (1909), for instance, gives 105 synonyms for yellow fever and his list can easily be augmented. BRYSON (1847) was the first to introduce some sort of order, so far as the ships of H.M. Navy were concerned, by allowing only three headings for fever in the naval returns—intermittent, remittent and yellow. As DUDLEY (1932) has pointed out, the first must have comprised chiefly benign tertian and quartan malaria and relapsing fever, the second largely subtertian malaria, with a variable amount of enteric fever and missed yellow fever cases. Blackwater fever also probably fell into this second group, together with occasional cases of leptospiral jaundice and epidemic catarrhal (common infective hepatic) jaundice. The last three diseases are hardly likely to have caused fulminating outbreaks with a high mortality, while the mildness of the enteric group of fevers in West Africa is well known. Epidemics with a heavy death rate either on shore or on board ship were therefore almost certainly due either to yellow fever or to malaria and even before the days of treatment by "the bark" the latter as compared with the former must have been a negligible cause of death. Moreover, deaths from cerebral malaria were, it appears, often entered as apoplexy or sunstroke.

The good example of the naval authorities in insisting on a standardized nomenclature was not, however, followed by the medical authorities ashore, and throughout the nineteenth and even into the present century deaths are said to have been due to "bilious haematuric" and "bilious remittent" fever.

Although therefore the earliest febrile epidemics with high mortality cannot with certainty be ascribed to yellow fever, there is a strong probability that they were due to this infection.

The Gambia was first visited by the Portuguese in 1455 when ALVICE DA CADAMOSTO entered the mouth of the river. In the following year at the instigation of HENRY THE NAVIGATOR he returned. While proceeding to the country of Battimansa, 60 miles up stream, fever broke out and one man was buried on a small island. At the end of the 11th day of his stay with Battimansa, CADAMOSTO decided to return to the mouth of the river as many of the crew had begun to suffer with a hot fever which was acute and continuous (RAMUSIO, 1563-83 and PRESTAGE, 1933). It is implied that if the sickness had not been so severe as to affect further progress it would not have been noted.

The next Portuguese expedition to reach the Gambia was also unfortunate. Either in 1456 or 1458 DIOGO GOMES ascended the river, accompanied by two caravels. One of these he left at Ollimansa, the other 50 leagues nearer the ocean, while he went further up to Cantor (the modern Kuntaur). Here his

men became worn out with the heat. He therefore returned in search of the other two caravels. In that which had remained at Ollimansa nine men had died, the captain, Gonçalves, was very ill and all the rest of the crew except three, were sick. In the other caravel five men had died. Unfortunately, information is lacking as to the total number comprising the crews of the two caravels, so that the death rate cannot be determined (PRESTAGE, 1935).

By the end of the sixteenth century the English were trading in the Gambia but the company founded in 1588 by QUEEN ELIZABETH did not prosper. In 1618 another trading company was incorporated and the *Catherine*, 120 tons, was sent out under Richard Thompson. With the exception of the captain the greater part of the crew of the *Catherine* was massacred. A second ship sailed in 1619, "arrived at an improper season" and lost most of the crew by sickness. At the end of the seventeenth century yet another corporation, "The Royal African Company," was founded and a fort was established on James Island in 1664. In the eighteenth century reports of the unhealthiness of the Gambia increase. STIBBS, on his arrival at Fort James in 1723, was greeted by the news that the Governor and two other Europeans had just died suddenly at Joar, one of the Company's factories. "Mr. Willy was buried on the North Bastion where several other Governors lie." MOORE (1738) himself suffered from an attack of fever while his assistant "died of a fever about the tenth day of his illness." MOORE notes that "while the ships lie in the River the Crews are apt to be sick and consequently not able to guard their slaves. . . . These musquetoos are the greatest plagues to one's person of any other vermin on the River. The musquetoos mind neither wind nor anything else but are always plaguing one, especially in the night."

After the middle of the century the treatment of fevers by quinine, "the bark," became more general, but there was no cessation of febrile epidemics with high mortality. LIND (1777), for instance (p. 172), quotes Mr. MARTIN, surgeon of the *Cataneuch*—a Guinea trader—to the effect that when he was in Gambia River in company with four other ships, "the men in one of those ships was daily taken ill of fevers and fluxes and several of them died delirious. Upon removing that ship about half a league from her first anchorage which was too near some swamps, her men became as healthy as those in the other ships." LIND (p. 196) also relates a very circumstantial account of an outbreak, said to have occurred in August, 1768, on board H.M. Sloop *Merlin*, "which continued six days in the river Gambia being employed in wooding and watering. While there, all the men were in perfect health but in about two days after they put to sea, those who had been employed in wooding were successively taken ill; afterwards those who had been employed in the duty of watering were seized in the same manner. Several of them in a day continued to fall sick for six or seven days afterwards, until at length almost all that had been employed on those services were ill: after them their attendants were seized with the fever and in such numbers as to leave no doubt of the disease being infectious." LIND

fails to mention whether any of these fevers were fatal and unfortunately the surgeon's journals for the period have now been destroyed. An inspection of the original log of H.M. Sloop *Merlin*, preserved in the Record Office, shows that LIND was not entirely correct in his facts. The *Merlin* did not visit the West Coast of Africa in 1768, as stated by LIND, but only arrived off Senegal from England on 1st July, 1769. On 14th July she anchored in the Gambia River and left again on 27th July for Sierra Leone. While off the latter port on 5th August "there departed this life Captain Thomas Male and Thomas Rogerman, surgeon's servant." These were the only fatalities on the voyage and this was the only visit of H.M. Sloop *Merlin* to the Gambia between 1766 and 1773.

A much fuller and more authentic account is given by ROBERTSON (1777) of an outbreak in H.M. Sloop *Weasel* in 1769 following a brief sojourn in the Gambia. "The ingenious Mr. Robertson," as LIND calls him, not only gives a very full account of the outbreak but by printing his daily journal enables the reader to follow accurately the course of the epidemic. The *Weasel* first sighted the Barbary Coast on her voyage from England on 22nd July and on the 28th was off the Gambia River. The next day she "stood in towards the River and spoke H.M. Sloop *Hound* and got a man out of her to pilot us up the River—he had a very sickly complexion"; 30th July was spent in getting up the River. "They anchored p.m." and next day the men were sent ashore to cut wood. "The men were exposed to the rains." On 1st August they continued up the River and ran aground near Fort James, "which occasioned the men's being very much fatigued in the heat of the sun." The following day they got the ship off and "after much trouble anchored in the sun nearly opposite the Fort." On 4th August "the men were employed in watering ship, the watering place being on the north bank at a point where it was very swampy and covered with trees and shrubs. The water was thickish, but had no bad taste. As boats could not get near the shore the men had to swim the casks off." On 7th August "most of the officers and gentlemen were ashore—which was all a marsh—shooting"; 8th August they got under way and began sailing down the river, being clear of it by 10th August.

Before the arrival of the *Weasel* in the Gambia there was very little sickness on board. On 30th July the *Hound's* man went sick within 24 hours of joining the ship. He suffered from a tertian intermittent fever and went back to duty on 14th August. Between 1st August and 14th August there was one bad case of tertian intermittent and eleven slight fevers which were very mild and easily

the patients complained of severe postorbital pains and were exceedingly dejected during the slight and temporary remission which often occurred about the 3rd or 4th day, to be followed later by delirium. On the 7th day some of their countenances were quite yellow and others looked wild. Vomiting and loose foetid stools were more general; the pulses were irregular. On the 8th day a few, after violent vomitings and purgings, broke out in purple blotches like the stinging of nettles, particularly about the face and neck, which soon disappeared. In one patient the parotid gland became swollen. On the 9th day "one who had purple blotches likewise had an hæmorrhage from the nose and mouth at times, his urine too was bloody." In one patient a large ecchymosis-like swelling appeared on the right side of the neck and face a little before death. The majority of those who died had never before been abroad. The following list of fatal cases, *taken from the ship's log*, shows that seven of the ten deaths occurred after an illness of less than 10 days duration. These figures differ slightly from those given in the text by ROBERTSON himself (p. 16) whence

FATAL CASES OF REMITTENT FEVER IN H.M. SLOOP *Weasel*, AUGUST, 1769.

Number of Cases.	Date of Onset.	Date of Death.	Number of Days Sick.
3	14.8.69	21.8.69	7
	20.8.69	27.8.69	7
	24.8.69	31.8.69	7
2	16.8.69	24.8.69	8
	20.8.69	24.8.69	8
2	17.8.69	26.8.69	9
	18.8.69	27.8.69	9
1	18.8.69	28.8.69	10
1	18.8.69	30.8.69	12
1	18.8.69	2.9.69	14

they have been copied (not quite accurately) by LIND (1777) and thence by CARTER (1931). They show that yellow fever cannot be so easily ruled out as a cause of the mortality as suggested by CARTER (p. 256). From 14th August to 27th August inclusive, thirty-three cases of remittent fever were treated, in a crew of ninety, though many of those who were not actually sick are said to have been far from well. On 27th August the ship was "smoked." One further case of fever occurred on 2nd September.

Indications which suggest that the disease may not have been yellow fever are, the fact that vomiting of matter like the grounds of coffee was not observed, and in one case a favourable crisis did not occur till the 18th day. "An eruption appeared about some of their mouths." CARTER (1922) regards herpes labialis

as common in malarial fever but very rare in yellow fever. However, five cases of herpes labialis have been observed among 800 persons immunized against yellow fever, so that its occurrence does not necessarily rule out yellow fever. The high mortality rate, the quick succession of deaths and the tendency to haemorrhage are in favour of yellow fever though almost certainly cases of malaria were also present on board. The fact that the outbreak ceased after fumigation of the ship is, however, highly suggestive evidence that during its stay in the Gambia the *Weasel* had taken on board mosquitoes infected with yellow fever, for if malaria had been the principal cause of sickness the destruction of the mosquitoes would hardly have caused so sudden a cessation of the outbreak.

In 1778 and 1779 cases occurred in the Gambia which were similar in character to those described by SCHOTTE (1780) in Senegal.

In the nineteenth century Bathurst becomes the chief centre of interest in regard to the incidence of yellow fever in the Gambia. Bathurst was founded in 1816, the greater part of the population being made up of liberated slaves with a small number of European officials and traders. Even by 1840 the population consisted of only thirty-six whites and 2,825 blacks. The first epidemic, almost certainly of yellow fever, occurred in 1825 when of a detachment of 108 men 74 died of a remittent fever. In 1828, according to BÉRENGER-FÉRAUD (1890) the whole coast from the Bight of Benin to the Gambia suffered from yellow fever but there is no record of cases actually occurring in Bathurst. The conditions in Bathurst in 1835 were very fully described by ALEXANDER (1837). Although many of the Europeans were then suffering from intermittent fever Mr. TEBBS, the surgeon, stated that at that time Europeans rarely died of fever. When he first arrived in 1829 the colonial surgeons had said to him "In bilious fevers you must not be surprised or annoyed if you lose one half of your patients. We consider ourselves lucky if we do not lose three fourths." They bled copiously and used mercurials. TEBBS ascribed his success to purgatives and the bark. Unfortunately, in the next epidemic which took place in 1837, TEBBS himself was one of the earliest victims. This outbreak was fully described by PYM (1848), and is noteworthy as being the first in the Gambia which was definitely described as yellow fever. H.M. Brig *Curlew* left Sierra Leone, where yellow fever was then epidemic, about the middle of May. While on passage the disease broke out among its crew and on arrival at Bathurst on 4th June fifteen men were either dead or dying. One half of the Europeans in the town succumbed, all those infected dying. Five Europeans, including the then Colonial Secretary, escaped to Senegal but all died of the disease, which then proceeded to carry off a large part of the population of Gorée.

Whether the disease was actually carried from Bathurst to Gorée is uncertain. Throughout the nineteenth century it was customary for the authorities of any infected port to accuse other ports of having passed on the infection. According

to BRYSON (1847) a fatal fever was already present in the Gambia in May, 1837, for H.M.S. *Fair Rosamond* in that month had sixteen cases, with five deaths. Details, however, are lacking as the surgeon himself died. Two persons who sailed in the *Rubis* to Gorée at the beginning of August are supposed to have infected that town. In 1842 four cases of yellow fever occurred among the garrison: two of the patients recovered. In August, 1859, what is described as "a lamentable epidemic" occurred. By the end of September only six Europeans were alive and some of these were convalescent. A year later (1860) three medical officers occupying the same dwelling at MacCarthy's Island "yielded to fever of a bad type after five days illness." In 1862 four cases of yellow fever were notified in Bathurst, two of the patients recovered. In 1866 Gorée and Bathurst again indulged in mutual accusations (CÉBONT, 1868). The former town had 249 cases with 110 deaths, the latter 17 deaths in the space of 4 months (July to October). In 1872 the prevailing diseases in Bathurst are said to have been yellow fever and smallpox, though exact details are lacking. BÉRENGER-FÉRAUD (1890) claims that he was instrumental in saving Gorée from infection though Bathurst was decimated at this time. In 1878 the inhabitants of Bathurst congratulated themselves on being not as other men, for whereas yellow fever was known to be raging in French territory 45 miles to the north and again 60 miles to the south, Bathurst itself was only visited by a "malarial fever." This malarial fever, however, was of such extraordinary malignancy and startling fatality that of the European population of between fifty and sixty, thirteen died, ten of them in the last quarter of the year. For the next 22 years there is no recorded epidemic of yellow fever, though occasionally Europeans died from "bilious haematuric fever."

In May, 1900, however, the "ancient tale of wrong" is continued and in the succeeding 5 months there were eleven cases of yellow fever with nine deaths. The outbreak is noteworthy, as it is for the first time recorded that "perhaps and not improbably in this case mosquitoes were the agents responsible for the yellow fever spreading from its original centre. It is worthy of note that all the cases (except one at the Catholic Mission) occurred in the front street of the town, the street most infested with mosquitoes." Following the report by DUTTON (1902) on conditions in the Gambia, sanitary reforms were instituted; though in 1903 a Catholic father died under somewhat suspicious circumstances from bilious remittent fever, no further epidemic occurred till 1911. In that year there were again eleven cases with nine deaths:—four cases with three deaths in May, six cases and five deaths in July, one case and one death in November. A full account of this outbreak is to be found in the *2nd Report of the Yellow Fever Commission (West Africa)*. Again there was a respite for 11 years, during which time, as a result of the War, the European population of Bathurst was greatly reduced. There was a new outbreak in October, 1922, when a young Moroccan died from yellow fever. Two mild cases were seen in adolescent Africans and two suspected cases also occurred. In November, 1928,

two Europeans and an African died from yellow fever and one African recovered : a month later a second African died and other cases were suspected. The first cases were associated with the premises of a trading firm heavily infected with *Aedes aegypti*. In October, 1934, there began the epidemic which is the subject of the present enquiry.

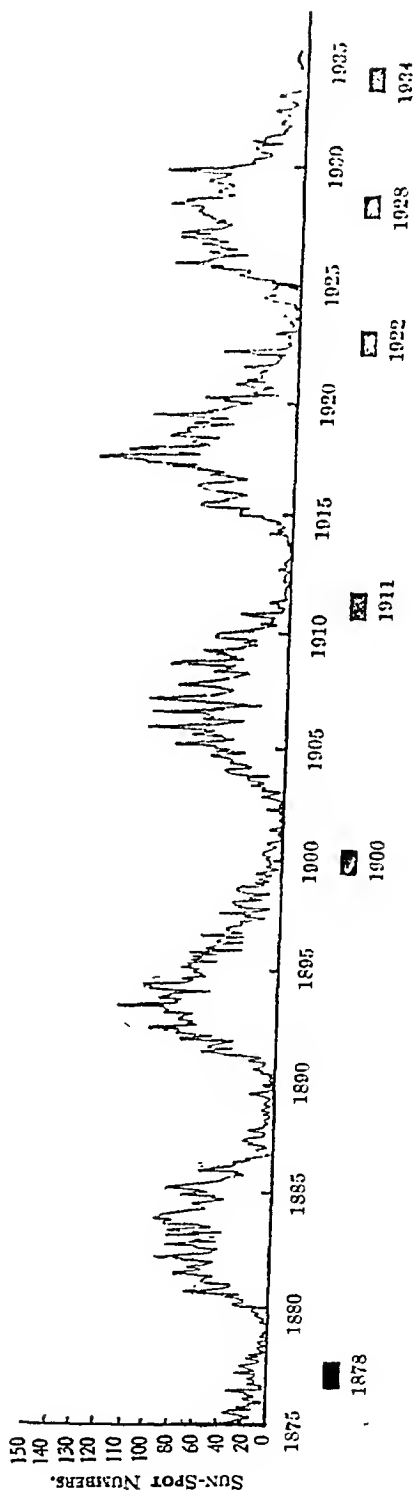
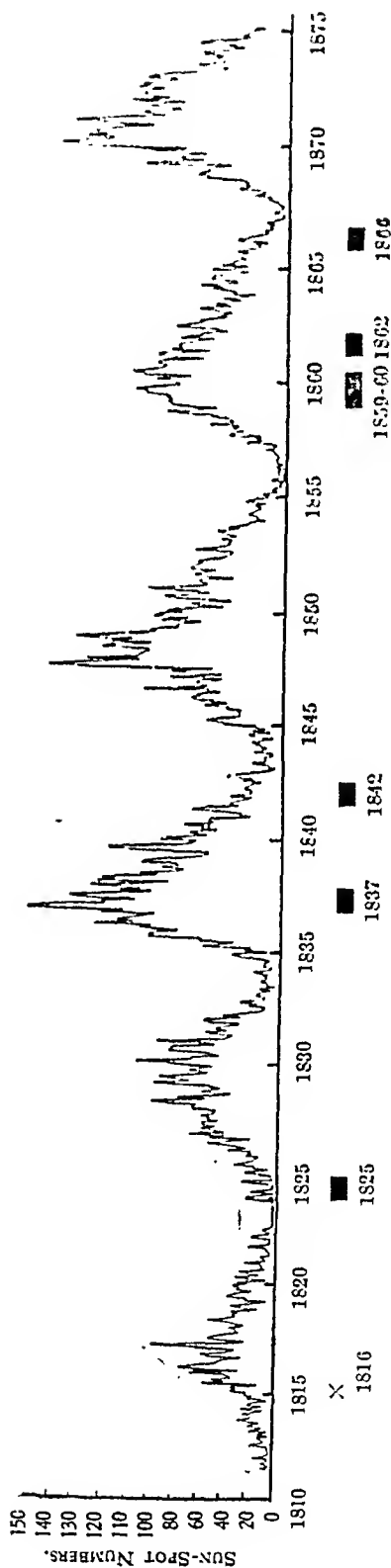
The history of acute and fatal epidemics among Europeans in the Gambia thus extends back for 480 years. Absolute proof that the earlier outbreaks were due to yellow fever is, of course, lacking, but in view of the uncertainty as to whether yellow fever is of African or American origin it is worthy of note that there was no change in the general character of the epidemics (i) either before or after the discovery of America, (ii) either before or after the institution of the slave trade, (iii) either before or after the general introduction of cinchona bark as a routine treatment for tropical fevers.

Since the foundation of Bathurst in 1816 it is noticeable that there is no history of any outbreak of yellow fever affecting the Africans in the Protectorate and no death in this area among Europeans except in 1860, when three medical officers are said to have died at MacCarthy Island. The Protectorate, in fact, might be regarded as " a silent area " for yellow fever.

So far as Bathurst is concerned, there is a very faint suggestion that epidemics of yellow fever tend to recur at intervals of either from 5 to 6 or from 11 to 12 years. The data, however, are of insufficient statistical value to establish any definite periodicity. DUTROULAU (1868), it may be added, found in the lesser Antilles a periodicity of either 6 or 10 years, while various authorities have suggested an 11 or 12 years periodicity for malaria epidemics. GILL (1936), as MELDRUM (1881) and others had done, has attempted to associate these malaria epidemics with variations in the sun-spot cycle, major epidemics occurring at periods of sun-spot minima, minor epidemics at sun-spot maxima, the average duration of the sun-spot cycle being just over 11 years.

The Chart on page 675 shows that from 1825 to 1935 there have occurred eleven periods of sun-spot minima. Eight of these periods have been associated with outbreaks of yellow fever in the Gambia. Of the four other yellow fever outbreaks in these 110 years, three occurred at or near periods of sun-spot maxima, while the outbreak of 1862 was probably merely a continuation of the 1859-60 epidemic.

It must not, however, be assumed that the sun-spot cycle and outbreaks of yellow fever are directly correlated. The sun-spot cycle is one of the twelve or more regular periodicities which together make up the cycle of solar variation. All the periodicities in solar variation are integral submultiples of 23 years ; as ABBOT (1935) has shown, the weather contains features which tend to repeat themselves at intervals of 23 years. Various phenomena depending on weather thus show the influence of the 23-year cycle—the level of the Nile, the levels of the Great Lakes, the rainfall of New England, the width of tree rings and the



SUN-SPOTS (AFTER ABBOT, 1935) IN RELATION TO OUTBREAKS OF YELLOW FEVER IN BATHURST, GAMBIA.

■ = Years when yellow fever was epidemic.
 X = Year in which Bathurst was founded.

abundance in the sea of cod and mackerel. Only further research can show whether periodic variations in mosquito populations also exhibit the influence of this cycle.

DISCUSSION.

The history here recounted of outbreaks of epidemic disease in the Gambia would seem at first sight merely to add further evidence to that so skilfully marshalled by CARTER (1932) in favour of the African origin of yellow fever. Since the publication of CARTER's book, however, many new facts have accumulated which render the problem of the original home of yellow fever still more complicated.

The following facts are in favour of the African origin of yellow fever :—

(i) As a result of the survey of Africa by the mouse protection test, Africans with immune bodies to yellow fever have been found as far east as the Bahr el Ghazal Province of the Anglo-Egyptian Sudan. There is evidence that the disease is not of recent introduction in this region.

(ii) The mild reaction of Africans to yellow fever suggests a long period of exposure to infection during which the more susceptible members of the indigenous population have been slowly eliminated and a racial resistance has been gradually acquired.

(iii) The failure of African monkeys to react to yellow fever virus by the development of clinical symptoms or pathological lesions also suggests a long period of exposure to infection.

(iv) *Aedes aegypti* is almost certainly an Old World mosquito since there are many parts of South America to which it has not yet penetrated.

On the other hand :

(i) Yellow fever has occurred in mountainous regions in South America far removed from the eastern seaboard.

(ii) A mild reaction to infection is not always met with in Africans since in certain outbreaks their mortality has been high. Inapparent infections are by no means rare in South Americans of mixed European and Indian descent while, as laboratory infections have shown, even persons of pure European descent may suffer from yellow fever in a very mild form.

(iii) Monkeys from East Africa where yellow fever has never been known to occur are no more susceptible than those from West Africa. West African monkeys are also relatively insusceptible to an East African virus, Rift Valley fever, to which they have never been exposed (FINDLAY, 1933).

(iv) Although there are many areas in South America where *Aedes aegypti* is absent yet the disease is effectively transmitted in these regions by indigenous South American mosquitoes such possibly as *Aedes scapularis*.

It is possible that a true explanation of the origin of yellow fever may eventually be found in the existence of an Old and a New World strain of yellow fever virus. The Old World strain, that transmitted by *Aedes aegypti*, has

undoubtedly crossed and recrossed the Atlantic on countless occasions in historical times, infecting and reinfecting the eastern seaboard of America. The New World strain would be that transmitted by non-domestic *Aedes* mosquitoes in the jungle regions of South America.

There is now growing evidence that yellow fever may persist in the absence of susceptible human beings while both in South America and in Africa (SOPER, 1935 ; and FINDLAY, STÉFANOPOULO, DAVEY and MAHAFFY, 1936) monkeys have been found with immune bodies to yellow fever. It is thus possible that yellow fever was present in Africa and in South America at a time long before the appearance of man. There is considerable evidence that at some remote period or periods the Old and New Worlds were united. Though WEGENER's theory of continental drift, which suggests that West Africa and Brazil were connected as late as the cretaceous period, has not met with universal acceptance since, as WATTS (1935) has pointed out, the resemblances between the tertiary floras of America and Europe actually increased at the time when, on the theory of continental drift, the Atlantic should have been widening, nevertheless the possibility of migration across polar lands or seas when terrestrial climates differed from the present cannot be ignored. SMITH WOODWARD (1935) for instance, suggests that the little Mesosaurian reptiles of the Permian rocks of both South America and South Africa, descendants of those found in the coal measures of North America and Europe, wandered south in parallel ways down the African and American continents. The ancestors of the primates and of the aedine mosquitoes may similarly have travelled south in two separate streams.

Evidence that two strains of virus may exist in a continuous land area is now forthcoming in the case of the virus of equine encephalomyelitis which in North America has differentiated into two strains, an eastern and a western form. The clinical symptoms and pathological changes produced by these two strains are practically identical while serologically the differences are quantitative rather than qualitative. The western strain, however, is readily transmitted by *Aedes aegypti*, the eastern with the greatest difficulty, if at all. Highly suggestive evidence of the existence of a New World strain of yellow fever virus might possibly be obtained by an examination of the bloods of Amazonian Indians from the still unexplored forests that lie to the north of Matto Grosso.

CONCLUSION.

Since the discovery of the Gambia in 1455, there have occurred among Europeans in this region periodic outbreaks of disease, attended by a high mortality.

These outbreaks occurred before and after the discovery of America, before and after the introduction of cinchona bark as a routine treatment of fever.

Since the foundation of Bathurst in 1816, outbreaks of yellow fever have occurred periodically in this town: they have not been recorded in the Gambia Protectorate.

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THE STUDY OF DISEASE PREVALENCE IN CHINA.

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It is an unfortunate fact that knowledge of medical and sanitary conditions of many of the greatest populations of the world is all too vague. Particularly is this true of Africa and Asia, where more accurate information of the occurrence of disease is urgently required. In this connection it may be of interest and importance to examine the methods that have been adopted in China for determining disease prevalence and distribution.

There are few medical problems which did not come under the purview of the Chinese ancients, and medical statistics are no exceptions. The following is an extract from WONG and WU (1932) :—

“The *Chou Rituals*, 10th century B.C., state that at the end of the year the work of the doctors is examined and the salary of each fixed according to the results shown. If the statistics show that out of the cases treated all get well, every satisfaction may be felt. If, however, 1 out of 10 dies, the results are only fair; if 3 out of 10 die, they are poor; if 4 out of 10, they are bad.” (*History of Chinese Medicine.*)

There is an immense Chinese medical literature, but its interpretation in terms of modern clinical and pathological nomenclature is extremely difficult. The classical medicine is inextricably interwoven with religious beliefs, astrology and demonology, and its philosophy governed by such ideas as that of the “Yang and Ying Principles” and the “Five Elements.” In most instances conclusions from such material are little more than conjecture. Nevertheless some facts have emerged, such as that clinical records were kept in the Han Dynasty, *i.e.*, about the time of Christ, by one TS’ANG KUNG, who described

twenty-five cases. After this, according to WONG and WU (1932), no further case histories are available until those recorded in the Sung Dynasty (A.D. 960-1297). Since that time writers in successive dynasties have given descriptive accounts of such conditions as eye diseases, leprosy, beri-beri, smallpox and syphilis, but apparently no attempts at formulating an index of the amount of disease were made.

The scientific description of disease distribution and prevalence in China had to await the permanent establishment of scientific medicine which dates from the founding of the dispensary for Chinese by LIVINGSTONE and MORRISON in Macao in 1820. In the years that followed, medical missionaries gradually spread throughout China until to-day there are few of even the remotest areas without some apostle of scientific medicine. Individual contributions to knowledge flowed in a steady stream from these pioneers who were stimulated, not only by the new and strange diseases of this great country, but also by contact with the medicine and herbal lore of the ancient Chinese civilization. Thus, indirectly, a rough pattern of the major diseases occurring in the missionary fields was woven, but the first concerted plan to study disease incidence and distribution came not from a missionary group, but from the Chinese Imperial Maritime Customs.

In 1860, the late Sir ROBERT HART, the Inspector General of the Customs Service, founded the Customs Medical Service with medical officers stationed at important Chinese sea and river ports. This was an outstanding landmark in the history of the collection of data of disease incidence in China, for the Service, from its inception, represented something more than a group of medical officers with limited official duties. It became an instrument of epidemiological research, which was a development of such extraordinary importance and evidence of such vision upon the part of its sponsors, Sir ROBERT HART and Dr. JAMIESON, that the circular initiating this function should be included amongst the classics of medical statistics. No apology need be made for reproducing it in this article on page 681.

Reports were issued over a period of 40 years, *viz.* 1870 to 1911, representing a standard of achievement that cannot be too highly admired and to-day the series is the essential introduction and preparation for epidemiological work in China. In them new diseases were reported, seasonal fluctuation noted and a rough description given of the occurrence of most of the major conditions.

In passing, it is interesting to record that numerous contributions by PATRICK MANSON illumined the pages of the Customs Medical Reports.

The band of Customs medical officers paved the way to an investigation of the types of disease prevalent in various parts of China and provided a mass of correlated data that is still yielding a harvest. Collective research, however, was not confined to them, in fact its brightest phase was yet to come in the service rendered by the medical missionary in support of his association.

As far back as 1887, in the first volume of the *China Medical Journal*, it was

INSPECTOR GENERAL'S CIRCULAR NO. 19 OF 1870.

INSPECTORATE GENERAL OF CUSTOMS,
PEKING.

31st December, 1870.

SIR,

1. It has been suggested to me that it would be well to take advantage of the circumstances in which the Customs Establishment is placed, to procure information with regard to disease amongst foreigners and natives in China; and I have, in consequence, come to the resolution of publishing half-yearly in collected form all that may be obtainable. If carried out to the extent hoped for, the scheme may prove highly useful to the medical profession both in China and at home, and to the public generally. I therefore look with confidence to the co-operation of the Customs Medical Officer at your port, and rely on his assisting me in this matter by framing a half-yearly report containing the result of his observations at upon the local peculiarities of disease, and upon diseases rarely or never encountered out of China. The facts brought forward and the opinions expressed will be arranged and published either with or without the name of the physician responsible for them, just as he may desire.

2. The suggestions of the Customs Medical Officers at the various ports as to the points which it would be well to have especially elucidated, will be of great value in the framing of a form which will save trouble to those members of the Medical profession, whether connected with the Customs or not, who will join in carrying out the plan proposed. Meanwhile I would particularly invite attention to:—

- (a) The general health of during the period reported on; the death rate amongst foreigners; and as far as possible, a classification of the causes of death.
- (b) Diseases prevalent at
- (c) General type of disease; peculiarities and complications encountered; special treatment demanded.
- (d) Relation of diseases to { Season.
Alteration in local conditions—such as drainage, etc.
Alteration in climatic conditions.
- (e) Peculiar diseases; especially leprosy.
- (f) Epidemics { Absence or presence.
Causes.
Course and treatment.
Fatality.

Other points, of a general or special kind, will naturally suggest themselves to medical men; what I have above called attention to will serve to fix the general scope of the undertaking. I have committed to Dr. R. ALEX. JAMIESON, of Shanghai, the charge of arranging the Reports for publication.

3. Considering the number of places at which the Customs Inspectorate has established offices, the thousands of miles north and south and east and west over which these offices are scattered, the varieties of climate, and the peculiar conditions to which, under such different circumstances, life and health are subjected, I believe the Inspectorate, aided by its Medical Officers, can do good service in the general interest in the direction indicated; and, as already stated, I rely with confidence on the support and assistance of the Medical Officer at each port in the furtherance and perfecting of this scheme. You will hand a copy of this Circular to Dr. and request him, in my name, to hand to you in future, for transmission to myself, half-yearly Reports of the kind required, for the half-years ending 31st March and 31st October—that is, for the winter and summer seasons.

I am, etc.,

(Signed) ROBERT HART, I.G.

The Commissioners of Customs:—

Newchwang

Tientsin

Chefoo

Hankow

Kiukiang

Chinkiang

Shanghai

Ningpo

Foochow

Tamsui

Takow

Amoy

Swatow and

Canton.

urged by a correspondent that missionary hospitals should place on record in the journal summaries of their annual reports. This suggestion bore some fruit, but it was not until the establishment of the Research Committee of the Chinese Medical Missionary Association that a comprehensive investigation of disease prevalence and distribution was planned. This early Committee in 1913 drew up an ambitious programme, but reference need be made here to one item only, eventually to be a most successful feature. This was a system of surveys. Faecal samples were collected in various parts of the country and the knowledge of the distribution of *Ancylostoma*, *Fasciolopsis* and other parasites thereby much extended. A further simple practical survey consisted in the preparation of blood smears which laid down the foundation of the present knowledge of the malaria problem in China. FAUST (1926), whose survey is now the most authoritative review of malaria in China, gives much credit to the pioneer work of the Association.

Other surveys of single disease groups have been undertaken, notably those by the Hookworm Commission (1926); of plague by WU, LIEN-TEH (1934) and his associates; and of schistosomiasis by FAUST and MELENEY (1924).

A further method of approach which was adopted by the Association is still being vigorously pursued, largely due to the efforts of J. L. MAXWELL (1930). He has consistently urged that missionary and other hospitals have a bounden duty not only to issue annual reports, but to use recognised disease nomenclatures and follow uniform classifications. As a result, realization of the importance of accurate recording is gradually developing and hospital reports from China will in time provide material of unusual assistance to statisticians and public health administrators.

The collective work conducted by the Customs Medical Service and by the China Medical Missionary Association forms the first comprehensive attempt to estimate the general prevalence of disease. Notice must also be given, however, to other accumulations of similar data, but restricted to local areas, made by two of the more important official health authorities in China, the Hongkong Government and the Shanghai Municipal Council. Hongkong was ceded to Britain by China in 1842 and shortly afterwards medical officers were stationed on the island, which, in its early days, had gained a bad reputation on account of the ravages wrought by malaria. With the evolution of a sanitary and health department came the issue of annual health reports for the colony: these continue to the present day and being based on a uniform model, they paint a picture of many medical happenings in South China which otherwise would not be available. It is possible to trace in them the introduction into that area of such diseases as rabies and foot-and-mouth disease, and to study the ebb and flow of such epidemics as plague and malaria.

However, the best municipal records of disease in China proper are undoubtedly the annual health reports of the Shanghai Municipal Council. Workers on such subjects as cholera, rabies, scarlet fever and veterinary conditions

find the only historical account of any reliability in the tables and text of these annual productions. That much of this material is available to-day is due to the efforts of Dr. J. H. JORDAN, Commissioner of Health, who, appreciating the importance of consecutive disease records saved much of the early literature from destruction. Official collections of morbidity and mortality statistics being only in their infancy in China, additional significance is given to the publications of these Hongkong and Shanghai organisations.

The sources of data thus far mentioned had their beginnings largely in the early days of scientific medicine in China and it is this historical range which renders them of the greatest value to the epidemiologist. The more accurate and specialised examination of disease incidence is a recent development. The first of these later studies to be mentioned is to the credit of an entirely Chinese institution, the National Epidemic Prevention Bureau. Accounts of the initiation of the intelligence system of this Bureau have been given by HUANG (1927) and also in a report published in 1934 by the National Health Administration. Communicable diseases, based on the list of the League of Nations Health Organisation, alone were considered, as naturally these were the main concern of the official machinery. Hospitals and physicians throughout the country were circularised and invited to return postcard accounts of infectious disease coming under their notice. The satisfactory response enabled the Bureau to issue monthly bulletins, which were also incorporated in the *Monthly Epidemiological Reports of the League of Nations Health Organisation*, but after a period of three years this function was, in 1928, transferred to the Central Government Department now known as the National Health Administration.

Extended and improved, this form of notification of communicable disease is now undertaken by the Department of Epidemiology and Vital Statistics, the standard postcard and monthly returns being supplied not only by hospitals in every province, but by the National Quarantine Service, and local health authorities. This is an important official work. Major communicable diseases such as plague, typhus, malaria and smallpox are thus to some extent kept under observation and their main movements followed and, for practical purposes, the essential data of epidemic control and prevention are provided. Quantitative results are not obtained, the postcard form indicating the degree and prevalence of disease, according to the reporting officers' opinion only by such terms as "sporadic," "prevalent," "epidemic" and "absent."

Consideration has now to be given to the latest methods of studying disease prevalence as applied in China. To appreciate the problem it is necessary to review the position of modern medicine in China, as it is only from scientific medical observers that estimates of disease can be expected.

Despite a century of effort, the new medicine is limited still to the towns, and as a generalisation it is true to state that the rural population, which forms 85 per cent. of the total population, is practically outside the observation of scientific medical practitioners, being under the influence of the herbalist and

follower of the ancient medical guilds. Even in the towns scientific medicine is but poorly represented, although some of the larger cities have municipal health services. There are the Departments of Health of the three local authorities in Shanghai, as well as those of Nanking, of Peiping, and of Canton. The National Government utilises as information agencies the Department of Epidemiology and Vital Statistics discussed above and the Quarantine Service representatives at the larger ports. Finally, there are the hospitals, which form the bulwarks of scientific medicine in China, and of an approximate total of 400 hospitals, over 250 are missionary institutions. A few of the leading hospitals, such as the Peiping Union Medical College Hospital, Peiping, the Central Hospital of Nanking, and the Municipal Hospitals of Shanghai are not under missionary control, but the great bulk of the efficient hospital institutions may be considered to be sponsored by some religious body. These hospitals are to be found in towns and cities throughout China, except in one or two provinces recently overrun by communists. There is very little scientific as opposed to ancient medical practice carried on apart from hospitals, as outside of the larger cities the general practitioner of modern medicine is unknown. Therefore, any system of disease-reporting has to be based on the use of the present machinery of modern medicine in China, in fact the hospitals.

The epidemiological aspects of disease are looming larger in China, and the need for guidance in the present attempts to establish medical and health programmes, both national and provincial, has directed attention to defining the extent and nature of disease problems. The method adopted by the National Epidemic Prevention Bureau has been outlined. When the First General Conference of the Chinese Medical Association met at Shanghai in October, 1932, the subject was designedly emphasised. Dr. EARLE, the Chairman of the Research Council of the Association, had come to appreciate the need for an ordered collection of disease statistics and as Director of the Henry Lester Institute of Medical Research, he had established a Department of Epidemiology and Medical Statistics with such a work in view. At the Congress he called upon Major P. GRANVILLE EDGE (1932) to deliver an address on the subject and this led to the adoption of a resolution that the Association undertake an investigation of the prevalence of disease in China as revealed by hospital patients, and that the Henry Lester Institute be responsible for the necessary organisation. In taking this decision, the Congress was quite aware of the deficiencies and errors that would be inevitable in an index based on hospital statistics. But, in the absence of an official vital and medical system for the country and the impossibility of its general establishment for many years, in the lack of general practitioners distributed through all classes and areas of the population, and in the absence of any other possible means of securing such evidence it was felt that the urgency of the question justified the use of hospital material.

Several factors have to be borne in mind in assessing hospital statistics. In China, hospitals are true general hospitals performing functions and treating

conditions more usually undertaken by general practitioners in other countries, but the hospitals are in urban areas and therefore give no picture of conditions in rural populations. Furthermore the Chinese in the mass do not appeal to the hospital for treatment in every instance of sickness or injury. They are apt to decide on each occasion whether the Chinese herbalist or the scientific doctor is the more likely to provide relief. It follows that, careful observers that they are, the Chinese have long discovered the superior merit of scientific surgery, quinine, salvarsan and many other treatments, but in some diseases they perceive that the herbalist is quite as effective (or non-effective) as the other. Likewise, questions of the hospital personnel, buildings, apparatus and treatment are also obviously related to the confidence and popularity inspired by scientific medicine and thereby produce varying degrees of success in different areas.

Hospital annual reports have been and are increasingly issued in China and many of them display in tabular form the conditions treated, but their construction, as in most parts of the world, lacks uniformity in disease terminology, classification, and definition of new and return cases. To the epidemiologist desirous of getting "a bird's eye view" of the nature of disease in China this defect renders hospital reports largely useless, and demonstrates again the need for serious consideration of a methodical analysis of morbidity statistics. To public health and to medicine, morbidity phenomena are more important than mortality forces but while the latter, through established international procedure, can be thoroughly described; the former have yet to be assessed by a standard measure of terminology and classification.

What was wanted by the Congress was an immediate disease "audit" to cover out-patient or clinic patients as well as the in-patients whose records alone are usually found in hospital reports. Accordingly it was arranged that representative hospitals from most of the provinces of China should complete an extremely simple card record of each new patient passing through their wards and clinics, these card records requiring the following items of information concerning each patient:—Age, sex, occupation, domicile, diagnosis. Instructions defining each item enabled uniformity to be approached.

During 1933 seventeen hospitals and during 1934 twenty-five hospitals forwarded each month cards of *all new in-patients and out-patients*. There resulted the collection of 474,654 card records by the department in the Lester Institute. This formidable mass of data has been and is still undergoing analysis, and reports have appeared in the *Chinese Medical Journal* as the different subjects were completed. For a full account these should be consulted, but the object of the present article is met by reference to the main features and conclusions alone.

On receipt of the cards the Chinese age was converted to Western standards by the accurate method introduced by STEVENSON (1926). The diagnoses, by reference to a modification of the International List of Causes of Death were classified under a Short Disease List of fifty causes. This classification had of necessity to be simple, as the returns made and the precision of diagnosis varied

those of the West. Instead of organizing the practitioners of scientific medicine into a united and progressive force, we have allowed ourselves to pursue the most wasteful line of individual competition. Lastly, instead of building up a system by which the most effective means of protecting the health of the people may be obtained, we have narrowed ourselves down to mere relief of suffering, very imperfect at that."

The movement is studying all aspects of rural welfare-literacy, agricultural methods, sanitation, disease, land tenure, etc., and its deliberations promise to be of profound import. However, only the question of disease-estimation can be referred to here. A simple system of birth and death registration has been evolved, school medical inspection is a routine procedure, dispensary and clinic records are collected and finally a health survey of a thousand families is in process of analysis. This last study is probably the most remarkable element yet revealed in the history of Chinese medical statistics. Such a sample cross-section of the general population alone portrays accurately the effects of ill-health and defect and when similar samples are taken throughout China a complete picture of disease prevalence will be drawn.

The example of Ting Hsien is being followed. The National Health Administration particularly is earnestly developing a similar experimental area in Chu-Yeng Hsien near Nanking. Other areas are those of the National Medical College at Kiachow near Shanghai, and the very important area which was early established by the Peiping Union Medical College at Peiping. This project, under the auspices of the Rockefeller Foundation has been among the most fruitful, and from it data are steadily accumulating on numerous subjects including those of vital and medical statistics. Both mortality and morbidity forces are being estimated. (GRANT, 1932).

For the present in China, public health administrators, hospital superintendents, directors of economic and social reform rely largely upon the unsatisfactory picture painted by hospital records of which that of the Chinese Medical Association is as yet the best attempt. Eventually they will be able to act boldly on the material provided by official statistics and sample surveys. China, in her active development of morbidity studies as opposed to mortality statistics, is taking a lead in methods of securing information essential to the guidance of health and medical movements, and as other countries increasingly appreciate the greater importance of morbidity over mortality statistics her methods will be imitated and followed.

SUMMARY.

1. The need for greater knowledge of disease distribution and prevalence in the larger world populations is stressed and the various methods of studying the problem in China are discussed.

2. The classical medical literature of China, though voluminous, is shown to give little clue to disease-occurrence through the centuries.

3. The first important effort to study the problem was represented by the Medical Reports of the Chinese Imperial Maritime Customs.

4. Estimations of disease prevalence by means of

(a) Surveys of individual diseases ;

(b) Insistence on scientific hospital reports,

had been actively undertaken by the China Medical Missionary Association.

5. In the absence of any general vital statistical service special significance had been lent to the Reports of the Hongkong and of the Shanghai Health Departments.

6. The realisation that hospitals alone could give the required information, there being no general practitioner service outside one or two of the larger cities, had resulted recently in the initiation of a Survey of Hospital Patients undertaken by the Chinese Medical Association. This Survey had collected half a million card records, the analysis of which had provided most valuable information.

7. A further development of great value in the study of medical statistical problems was the use of Experimental Health Areas, such as those of the National Health Administration, the Mass Education Movement at Ting Hsien, and the Peiping Union Medical College Special Health Area.

8. Finally, in the emphasis she has given to morbidity statistics China has given a lead to those countries which still rely too much on mortality data for guidance in medical and health policies.

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CORRESPONDENCE.

ANTELOPES AS RESERVOIRS OF *TRYPANOSOMA GAMBIENSE*.

To the Editor, 'TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene.'

SIR,

BRUCE and his co-workers in 1910 made some well-known experiments with *Trypanosoma gambiense* and antelopes. The antelopes were caught within a distance of two or three days' journey from the laboratory at Mpumu. Although a preliminary inoculation of their blood was made into monkeys or rats, it will probably have seemed to many persons who have read the reports carefully that the existence of a chronic infection of the antelopes with *T. brucei* was not excluded. There seems to be little doubt that some at least of the antelopes became infected with *T. gambiense*, but whether the trypanosomes which were transmitted from the antelopes several months (in one case 22 months) afterwards were *T. gambiense* or *T. brucei* appears to be doubtful as no experiments were made on human beings. It has been repeatedly shown that *T. gambiense* can live for many months in laboratory animals and in sheep and goats, though individual animals, even monkeys, may resist infection and, if infected, may recover. It is not improbable therefore that the conclusions of BRUCE and his co-workers were right, but the importance of the subject makes it desirable that the experiments be repeated under stricter conditions, even if it is necessary to make them on a smaller scale. The possibility of the existence of the animal reservoir needs to be demonstrated before attempts to estimate its practical importance are made. The opinion of members of that Commission who are still alive and remember the local conditions would be useful. In references to that work that have appeared from time to time, the identity of the trypanosome has rarely, if ever, been questioned. *Trypanosoma brucei* was not found in the neighbourhood at the time, but it is hard to see how a mild strain could have been distinguished from *T. gambiense* without a test on man.

I am, etc.,

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